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8707 C*

Low Concentrations of Deuterium Oxide and the Growth of Marine Diatoms.

Denis L. Fox, Easter E. Cupp and George F. McEwen.
From the Scripps Institute of Oceanography, University of California, La Jolla.

An early suggestion was made by Barnes and Jahn[1] that if slight increases in the concentration of the heavy isotope of water should occur through fractionation during the slow sublimation of ice and snow masses, the extra traces of D_2O might help to explain the increased reproductive activity of organisms in waters from frozen sources during the spring of the year. In view of this interesting suggestion, and because of the very contradictory reports regarding stimulative, retarding or inhibitory effects, or complete lack of influence of heavy water solutions upon growing organisms or enzyme systems (*cf.* Fox[2]), it seemed of interest to investigate carefully the relative growth rates of some isolated cultures of marine diatoms in nutrient sea water alone and in the same medium to which had been added small amounts of (1) ordinary twice-distilled water, and (2) highly pure D_2O.

Fresh sea water, collected from the ocean in glass vessels, was passed through a Berkefeld filter candle of fine porosity; modified

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* P represents a preliminary, C a complete manuscript.

[1] Barnes, T. C., and Jahn, T. L., *Quart. Rev. Biol.*, 1934, **9**, 292.

[2] Fox, D. L., *J. Cell. and Comp. Physiol.*, 1935, **6**, 405.

Miquels' solution was prepared and added (Allen and Nelson[3]) and the final solution was heated to about 70°C. for 20 minutes, allowed to cool, and used as the culture medium.

Nitzschia bilobata var. *minor* Grun. and *N. closterium* (Ehr.) W. Sm., the 2 species investigated, were isolated from sea water at La Jolla, and flourishing cultures were grown free from other algae, fungi, and protozoa, but no attempt was made to render them bacterially sterile.

The control series (designated as C in the tables) contained the organisms in the undiluted culture medium described; the second type of control (H) contained one percent of added twice-distilled ordinary water; and the D series contained instead one percent of added 99.2% D_2O.†

Into each of the series of Pyrex Erlenmeyer flasks of 50 ml. capacity were introduced 10 ml. of the media; 0.1 ml. of the diatom culture was then delivered from a Mohr pipette into each vessel. All flasks were covered with glass plates (*N. closterium* series) or stoppered with cotton plugs (*N. bilobata* series) to retard evaporation during the course of the experiments. Both series of flasks were placed in straight rows upon a rack before a window with northern exposure; they received only diffused daylight, and care was exercised in placing them in such order that each member of successive groups occupied alternate relative positions with respect to one another (*i. e.*, C_1—H_1—D_1; H_2—D_2—C_2; D_3—C_3—H_3, etc.). The original inoculation of the respective culture series was conducted in the same relative sequence in order to secure as nearly as possible the same conditions, on the average, for each series.

With uniform conditions of light, temperature (room), relative quantities of solution and air in each vessel (all of which were of the same shape) and with care used in the selection and sampling of organisms which, at the stage of transfer, did not form persistent clumps, we considered that we had circumvented the common sources of avoidable error.

At stated intervals of days, the cells in a set of flasks from each kind of culture in the series, were killed by the addition of a little formalin (*i. e.*, a virtually constant volume of 4-5 drops added to each volume of 10.1 ml. culture, giving a uniform change of only about 2%) and set aside to be counted after the dead cells should

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[3] Allen, E. J., and Nelson, E. W., *J. Marine Biol. Assn. of Utd. Kgd.*, 1910, **8**, 421.

† This heavy water of certified high purity was purchased from the Norsk Hydro-Elektrisk Kvaelstoffaktieselskab, Oslo, Norway.

TABLE I.

Growth of *Nitzschia Bilobata* var. Minor Grun.

Days of Growth	No. of Cells per ml.		
	H	C	D
0	9	9	9
3	984	1278	426
	1206	678	576
	1518	1368	402
	3078	2508	1704
6	3768	2526	2520
	4734	3650	3150
	3972	3426	2274
	3372	2484	2202
9	6222	5310	4194
	5136	4260	4518
	4674	4866	2886
	5220	4722	4488
12	4332	5718	4320
	4680	4278	4092
	4956	4098	4362
	5334	4440	3888

Average cell length 63μ; width at widest portion of valve 5.5μ.

H—contained 1% of ordinary twice distilled water.

C—unaltered.

D—contained 1% of 99.2% D_2O.

Number of cells added initially to each flask (average of 8 counts, viz.: 81, 85, 85, 92, 97, 105, 96, 82) = 90 cells per 0.1 ml. or 9 cells per ml. of culture.

TABLE II.

Growth of *Nitzschia Closterium* (Ehr.) W. Sm.

Days of Growth	No. of Cells per ml.		
	H	C	D
0	368	368	368
3	21,210	71,400	94,536
	11,550	45,000	71,280
	14,280	95,280	60,048
Aver.	15,680	70,560	75,288
5	138,600	71,040	324,144
	180,720	186,720	364,032
	303,360	264,960	325,440
Aver.	207,560	174,240	337,872
7	511,200	497,088	527,040
	662,400	475,200	471,456
	514,080	331,200	569,376
Aver.	562,560	434,496	522,624

Aver. length 57μ (length of region occupied by chloroplast 23μ); width at widest region 4.5μ.

Number of cells added initially to each flask (average of 6 counts, viz.: 342, 312, 336, 498, 312, 408) = 368 cells per ml.

have become disengaged from clumped aggregates. Counting was conducted with the use of a Sedgwick-Rafter counting cell, in accordance with standard procedure. Tables I and II present the experimental data.

Although a general decrease in the number of cells per ml. with respect to "treatments" H, C, D, is indicated in Table I, the high variability between experiments within each group and the lack of complete uniformity in the indications requires special consideration. For this purpose the statistical method of analysis of variance introduced by Fisher and Mackenzie[4] was applied. For an elementary explanation of this method and illustrative problems, see Snedecor.[5] With reference to this experiment, see especially his illustrative example 6, pages 40-45. Essentially, analysis of variance is a statistical technique for isolating the effects of known factors on the variability and estimating the residual experimental error. By calculating the variance or square of the standard deviation of the observations, grouped according to each of the known factors, and comparing each variance with the residual, and using Fisher's probability tables, its statistical significance can be determined. The computations relative to Table I are outlined in Table III. The residual or mean variance within classes, 417,000, is a measure of the experimental error. The standard deviation of one observation and of the difference between the averages of 4 observations have the respective values $\sqrt{417{,}000} = 645.0$ and $(\sqrt{2}/\sqrt{4})\ 645.0 = 456$, which can be used in the usual way for testing the significance of any differences. For testing the composite results of all differences, use the ratio of variance between means of treatment classes to the residual. The large value, 11.23, indicates a level much less than 1%, which is highly significant. The low value of the interaction, 46,849, as compared to the residual, indicates a very small differential in the response to treatment at different times; thus corresponding to a very consistent result.

The same method applied to the observations in Table II showed a consistent response to the treatment, but a relatively high experimental error corresponding to a 5% level, which, although suggestive, is barely significant.

Ewart[6] reports that very dilute heavy water (1 to 1.3 per 1000) seemed to exercise a retarding action upon the metabolism of non-

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[4] Fisher, R. A., and MacKenzie, W. A., *Agricultural Science*, 1923, **13**, 311.

[5] Snedecor, G. W., Variance and Covariance. 105 pages, Collegiate Press, Inc., Ames, Iowa. 1934.

[6] Ewart, E. J., *Austr. J. Exp. Biol. and Med. Sci.*, 1935, **13**, 9.

TABLE III.
Analysis of Variance of Counts of *Nitzschia Bilobata* var. Minor Grun.
Number per Sample and Number of Cells per Liter or Growth of Culture.

Time	H No.	H Growth	H Mean	C No.	C Growth	C Mean	D No.	D Growth	D Mean	Totals No.	Totals Growth	Totals Mean
3	4	6,788	1,697	4	5,832	1,458	4	3,108	777	12	15,728	1,312
6	4	15,848	3,962	4	12,088	3,022	4	10,148	2,537	12	38,084	3,175
9	4	21,252	5,313	4	19,160	4,790	4	16,088	4,022	12	56,500	4,710
12	4	19,304	4,826	4	18,536	4,634	4	16,664	4,166	12	54,504	4,542
Totals	16	63,192	3,949	16	55,616	3,478	16	46,008	2,877	48	164,816	3,436

SUMMARY OF COMPUTATIONS.

Source of Variation	Total	Within Classes	Between Means of Time Classes	Between Means of Treatment Classes	Interaction
Variance	113,511,404	15,058,844	89,134,887	9,270,824	46,849
Degrees of Freedom	$48 - 1 = 47$	$(48 - 1) - (12 - 1) = 36 = N_2$	$4 - 1 = 3$	$3 - 1 = 2$	$3 \times 2 = 6$
Mean Variance	2,419,000	417,000	29,700,000	4,637,000	7,810

RATIOS OF VARIANCES

	Ratio corresponding to 1% level
$29{,}700{,}000 \div 417{,}000 = 71.2$	4.40
$4{,}637{,}000 \div 417{,}000 = 11.23$	5.27

$\sigma = \sqrt{417{,}000} = 645.0$

$\frac{\sigma\sqrt{2}}{\sqrt{4}} = 456$ Standard deviation of difference between means of 4.

chlorophyllous plants in general, while it showed an accelerating effect upon chlorophyllous plants, or upon organisms without chlorophyll (yeast) or seeds of green plants (oats) whose dominant function is carbohydrate metabolism.

Curry and Trelease[7] found that the evolution of oxygen during photosynthesis by *Chlorella* in pure buffered D_2O was 0.41 of that in the ordinary water buffered controls. They cite the work of Reitz and Bonhoeffer,[8] who calculated on the basis of chemical analysis velocity constants for the assimilation of both hydrogen isotopes by algae cultured in D_2O and in ordinary water, finding the ratio of the constants to be about $K_D/K_H = 0.43$. Fox (op. cit.) and Fox and Craig[9] found that starch in which some of the H had been presumably exchanged for D was somewhat more readily hydrolyzed by an animal amylase. (See also similar results of Macht and Bryan.[10]) Thorough studies of many such relatively isolated biochemical systems would seem to be the proper approach to the possible influences of heavy water upon complex integrated processes of metabolism.

Summary. A consistent lag of about 16% (quotient of total mean into D mean, Table III) in the growth rate of *Nitzschia bilobata* in the dilute heavy water is unmistakably apparent from a survey and mathematical analysis of the data in Table I.

The data in Table II show a trend of the opposite character, particularly in the earlier times, but are not sufficiently numerous for a conclusive interpretation. Any effects must, therefore, be very slight.

That heavy water may be without detected physiological effects, or may exert influences of either a stimulative or retarding character is certain, but the situation is somewhat analogous to that of the hydrogen ion concentration in that the nature and extent of observed influences depend upon the organism or other biochemical system under investigation, the concentration of heavy isotope, and probably a host of other physical and chemical factors.

Gratitude is expressed to the members of the 1934-35 Committee on Grants-in-Aid of the National Research Council, whose generosity toward the senior author's project made possible the acquisition of the heavy water.

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[7] Curry, J., and Trelease, S. F., *Science*, 1935, **82**, 18.

[8] Reitz, O., and Bonhoeffer, K. F., *Z. physik. Chem. A.*, 1935, **172**, 369.

[9] Fox, D. L., and Craig, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 266.

[10] Macht, D. I., and Bryan, H. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 190.

While the problem arose in the physiological laboratory where other research on heavy water was being carried on, the identification and cultural isolation of the diatoms were the work of E. E. Cupp, of the Phytoplankton section here; she also made the population counts. G. F. McEwen, of the section of Physical and Dynamic Oceanography, performed the mathematical analyses and wrote the discussion of their significance.

8708 C

The Effect of Filtrates of *Cl. histolyticum* upon the Growth of Animal Tumors.

HAROLD P. RUSCH* AND WILLIAM S. PRESTON. (Introduced by Walter J. Meek.)

From the Departments of Physiology and Medical Bacteriology, University of Wisconsin.

Connell[1] has recently reported a small series of cancer patients who, he believed, were benefited by the use of proteolytic enzymes, which were obtained by the growth of *Cl. histolyticum* upon tumor tissue. Torrey and Kahn[2] had previously produced an enzyme by the growth of *Cl. histolyticum* on a 3 to 4% peptone meat infusion broth and following the injection of this material into the Flexner-Jobling rat carcinoma, reported a 50 to 75% cure. Parenteral injections were of no value. Des Ligneris,[3] in quoting some earlier work of his along similar lines, reports results of an entirely negative character. Gye[4] was unable to confirm Connell's results, using mice as test animals.

The experiments here reported were done for the purpose of testing the effect of certain bacterial filtrates prepared according to the method of Connell upon the rate of growth of 2 animal tumors, whose characteristics are well known and thoroughly established. According to Connell's statement, such solutions should contain proteolytic enzymes.

Preparation of the bacterial filtrates. Three types of tissues were used separately as sources of media for the production of the filtrates: the viable portions of the Flexner-Jobling rat carcinoma, mouse sarcoma No. 180, and rat muscle, the latter serving as a control. These tissues were removed with aseptic precautions, and about 15 gm. of each tissue placed in separate Erlenmeyer flasks to which normal saline was added in the proportion of about 10 cc. to one gram of the tissue. The media were then inoculated with a pure culture of *Cl. histolyticum* and incubated at 37.5°C. under anaerobic conditions for from 4 to 6 days until the contained tissue had become disintegrated. The liquid contents of the culture were then filtered first through filter paper and then through Mandler candles, following which each was placed in a sterile container and kept in the refrigerator until used.

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*Jonathan Bowman Cancer Research Fellow.

[1] Connell, Hendry, *Canadian Med. J.*, 1935, **33**, 364.

[2] Torrey and Kahn, *J. Cancer Research*, 1927, **11**, 334.

[3] Des Ligneris, *Brit. Med. J.*, Dec. 28, 1935, p. 1280.

[4] Gye, *Brit. Med. J.*, Oct. 19, 1935, p. 760.

The 3 different solutions obtained by this procedure will be referred to as "rat tumor filtrate," "mouse tumor filtrate," and "rat muscle filtrate." These solutions were given by one of 3 routes: intramuscularly (thigh muscles), intraperitoneally, or directly into the tumor in 0.5 cc. doses daily in rats and 0.2 cc. amounts every other day in mice, continuing until the animals died. In preliminary work it was found that larger, more frequent doses in mice would result in a loss of weight and early death due to toxicity. The rat tumor filtrate was given to rats and the mouse tumor filtrate to mice, except for 6 animals of each species, where cross injections were performed to test for specificity of the solutions. The rat muscle filtrate injected into the thigh muscles of the rats also served to detect any proteolytic action on normal tissue. In the rats injected directly into the tumor, only animals were used which had previously been inoculated with tumor material in 2 sites. Injections were made into one tumor with the filtrate and with normal saline into the opposite one.

Injections were begun only after the inoculated tumor cells were apparently established and by the size of the growing mass it could be more readily determined which tumors would be likely to grow and not regress spontaneously. This was about a week after the inoculation in the mice and about 3 weeks in the rats. All tumors were measured at weekly intervals. An inbred strain of mice, "C-57" (black) of Little, susceptible to sarcoma No. 180, and a closely inbred strain of stock rats having about an 85% susceptibility to the Flexner-Jobling carcinoma were the animals used.

In vitro tests were done to test for proteolytic enzymes and for any specificity which they might have. Pieces of rat tumor, mouse tumor, and rat muscle were removed with aseptic precautions and placed in test tubes to which 5 cc. of one of the 3 filtrates was added. Five tubes were used for each test. These were then

TABLE I.

Animal used	No. animals in exp.	Source of bacterial filtrate	Site of injection	Amt. injected	Average length of life (days)
Rat	10	Flexner-Jobling rat carcinoma	Into tumor	0.5 cc. daily	28
,,	19	,, ,, ,, ,,	Intramuscularly	,,	44.8
,,	15	Normal saline solution	,,	,,	50
,,	6	Flexner-Jobling rat carcinoma	Intraperitoneally	,,	40
,,	6	Normal saline solution	,,	,,	45
,,	5	Rat muscle	Intramuscularly	,,	38
,,	6	Mouse sarcoma No. 180	,,	,,	41
Mouse	15	,, ,, ,, ,,	Into tumor	0.2 cc. every other day	9
,,	15	Normal saline solution	,, ,,	,,	7
,,	19	Mouse sarcoma No. 180	Intramuscularly	,,	11
,,	18	Normal saline solution	,,	,,	10.1
,,	18	Mouse sarcoma No. 180	Intraperitoneally	,,	5.3
,,	15	Normal saline solution	,,	,,	12
,,	6	Flexner-Jobling rat carcinoma	Intramuscularly	,,	9

placed in an incubator for one week and observed for tissue disintegration at the end of this time.

No inhibition in the rate of tumor growth was noted in any of the treated animals as compared with the controls except when the mouse tumor filtrate was given intraperitoneally to mice. Following this procedure, the tumor growth was halted, the mice lost weight, appeared toxic, and died in a few days. That the solution was toxic for normal animals was demonstrated by the fact that when somewhat larger doses, 0.4 cc., were given intraperitoneally to healthy mice, the animals died in from 2 to 6 days. No difference in the number and frequency of metastases were noted in the treated and control animals.

The variation in length of life noted in the different experiments, except in the group mentioned in the preceding paragraph, is not considered of any significance, because of the small number of animals used in each series.

The early breakdown and necrosis which occurs in a tumor following the injection of many types of solutions directly into it appeared to be much more severe in the tumors in which the solutions were given as compared with those in which normal saline was used. However, the tumors continued growing at parts not reached by the solution, and this finding could be determined only, in degree, by comparative observation.

Sections of thigh muscles which had been injected with the solutions used were removed at autopsy and examined for histological changes. While evidence of inflammation was present in almost all, and infection in several, no evidence of a proteolysis could be observed by this method.

In the *in vitro* tests for proteolysis, none of the tissues suffered a disintegration such as that noted in those in which *Cl. histolyticum* is growing. Only softening of the tissues in varying degrees was noted. This was probably due to autolysis, since it was not more pronounced than that seen in the saline controls. According to this method of testing, we were unable to demonstrate any enzymatic activity in the filtrate.

Conclusions. The solutions prepared according to the method of Connell and tested upon mouse sarcoma No. 180 and the Flexner-Jobling rat carcinoma were without any effect in prolonging the life of the animals, in preventing or decreasing the number of metastases or in inhibiting the rate of tumor growth.

NOTE: Since this paper was written, Pommerenke (*J. Am. Med. Assn.*, 1936, **106**, 1654) has reported results of a negative character in the use of similar bacterial filtrates tested upon the Brown-Pearce rabbit epithelioma.

8709 P

Significance of Early Appearance of Neutralizing Substances in Poliomyelitis of Man.*

PAUL H. HARMON, HENRY N. HARKINS, JOHN J. FAHEY AND
PETER M. WASBOTTEN.

From the Division of Orthopedic Surgery, the Department of Surgery, University of Chicago.

Although it has long been known that the serum of patients convalescent from acute anterior poliomyelitis contains an antiviral substance, the fact that a similar substance is present in the blood of the majority of normal adults has been recognized only in recent years.[1, 2] By analogy to the known subclinical acquisition of immunity against diphtheria, the assumption has been made that a similar mechanism is operative in poliomyelitis. Such a belief postulates wide dissemination of the virus with a minor incidence of actual paralysis. Endocrine factors have been suggested in certain recent experiments.[3] However, another group of investigators[4] has been unable to reproduce the data of the first group. Further, the exact rôle that the poliocidal substances play in preventing infection under natural conditions and in recovery from the disease has not been elucidated, it having been assumed that these substances are the humoral expression of the immune state. One of us,[1] in collaboration with others, showed that occasional samples of convalescent human serum were devoid of neutralizing substances, while Howitt[5] found 80 of 141 samples taken weeks or months after an acute attack to be likewise without these substances. She attributed her findings to the fact that many of her patients received convalescent serum therapy.

In an effort to obtain further information upon the behavior of

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* Supported by a grant from the President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Shaughnessy, H. J., Harmon, P. H., and Gordon, F. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 742; *J. Prev. Med.*, 1930, **4**, 463.

[2] Aycock, W. L., and Kramer, S. D., *J. Prev. Med.*, 1930, **4**, 189, 201; Fairbrother, R. W., and Brown, W. G. S., *Lancet*, 1930, **2**, 895; Schultz, E. W., and Gebhardt, L. P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 409; Faber, H. K., *J. Am. Med. Assn.*, 1931, **96**, 935; Brodie, M., *J. Exp. Med.*, 1932, **56**, 507; Kolmer, J. A., *J. Am. Med. Assn.*, 1935, **105**, 1956.

[3] Jungeblut, C. W., and Engle, E. T., *J. Immunol.*, 1933, **24**, 267; *J. Exp. Med.*, 1934, **59**, 43; *J. Am. Med. Assn.*, 1932, **99**, 2091.

[4] Hudson, N. P., Lennette, E. H., and King, E. Q., *J. Exp. Med.*, 1934, **59**, 543.

[5] Howitt, B. F., *J. Infect. Dis.*, 1932, **51**, 574; Personal communication.

the poliocidal substances following an acute attack of the disease, we have titrated the serums from patients obtained as soon as possible after the onset of the acute disease and again after several months. Each of these serums has been mixed *in vitro* in several dilutions with 10 paralyzing doses of the virus (PMV) contained in a one percent centrifuged emulsion of glycerolated spinal cord removed from monkeys *(Macaca mulatta)* at the height of paralysis. Injection of the serum-virus mixtures intracerebrally into unused rhesus monkeys was the indicator of unneutralized virus.

The results of the experiments show that a majority of serums (12 of 14) contain the neutralizing substance quite early. Indeed, one patient, an adult, had a greater than average quantity of these substances even before becoming paralyzed. This observation appears to be unique in that a person with poliocidal substances in the blood subsequently developed paralysis. Additional observations will have to be made to determine if this is a regular or an exceptional phenomenon. Collateral determinations by the same serological technique on the serums of monkeys at various intervals during convalescence from the experimental disease have shown that poliocidal substance is not demonstrable until the ninth to fourteenth day after the onset of paralysis and then only irregularly. In this light, the interpretation of the data assumes special significance. Although 8 of the 14 serums were taken 1 to 3 days after pooled convalescent human serum was given in treatment, it does not appear likely, in view of the known low titre of such serums[6] that this quantity of poliocidal substance has been solely produced by passive transfer. Since these were all instances of sporadic poliomyelitis in a non-epidemic year, it is possible that these substances had been produced during a long incubation period. Against this assumption is the observation, the first upon record, of poliocidal substance being present in a patient in the preparalytic stage.

Comparison of the titre of the neutralizing substances several months later showed one patient with none (under the conditions of these tests), many with a quantity identical with the first titration and only a few (4 of 11) in which an actual increase was seen. These observations taken in conjunction with the inability of Kramer and Aycock[7] to demonstrate an increase in the incidence of these substances in a child population from a community where abortive and clinical poliomyelitis was prevalent, casts doubt upon the importance of a single attack of poliomyelitis for the production of antiviral substance.

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[6] Hudson, N. P., and Lennette, E. H., *J. Prev. Med.*, 1932, **6**, 335.

[7] Kramer, S. D., and Aycock, W. L., Proc. Soc. Exp. Biol. and Med., 1931, **29**, 98.

8710 C

Oxygen Utilization, Cardiac Output, and Related Circulatory Functions in Graves' Disease.

SIDNEY A. GLADSTONE. (Introduced by Edward Tolstoi.)
New York City.

The increased metabolic and circulatory rates in Graves' disease have been subjected to repeated quantitative study. The relation of these two functions was indicated by Fick in 1870 and may be expressed as follows:

$$\begin{array}{c}\text{Cardiac Output}\\ \text{(liters of blood per min.)}\end{array} = \frac{\text{oxygen consumption (cc. of oxygen per min.)}}{\begin{array}{c}\text{arterio-venous oxygen difference}\\ \text{(cc. of oxygen per liter of blood)}\end{array}}$$

Although previous workers have uniformly reported an abnormal increase both in the cardiac output and the rate of oxygen consumption, there has been a definite disagreement regarding the arterio-venous oxygen difference, which represents the number of cc. of oxygen taken up by each liter of venous blood during its passage through the lungs, or the average number of cc. of oxygen given off by each liter of arterial blood during its passage through the several tissues. It is the arterio-venous oxygen or A-V difference which is measured by the various foreign gas methods subsequently mentioned, the cardiac output being calculated from the quotient indicated above. Thus Liljestrand and Stenström,[1] using the foreign gas method of Krogh and Lindhard, and Bansi,[2] using the indirect Fick methods of Haldane and Christenson and of Redfield, Bock, and Meakins found the A-V (arterio-venous oxygen) difference decreased below normal. Fullerton and Harrop[3] and Boothby and Rynearson,[4] using the foreign gas method of Marshall and Grollman found no significant change in the A-V difference in Graves' disease as compared to normal. It is important to attempt to settle this disagreement for 3 reasons. First, the cardiac output is indirectly proportional to, and is calculated from, the A-V difference. Secondly, the normal or abnormal utilization of oxygen in the tissues may throw light on the nature of the metabolic disturbance in Graves' disease. Thirdly, the settlement of this disagreement may indicate the comparative reliability of the methods used in determining the arterio-venous oxygen difference and cardiac output.

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[1] Liljestrand, G., and Stenström, N., *Acta Med. Scand.*, 1925, **63**, 99.

[2] Bansi, H. W., *Z. für klin. Med.*, 1929, **110**, 633.

[3] Fullerton, C. W., and Harrop, G. A., *Bull. Johns Hop. Hosp.*, 1930, **46**, 203.

[4] Boothby, W. M., and Rynearson, E. H., *Arch. Int. Med.*, 1935, **55**, 542.

In the present study, the A-V difference and cardiac output have been determined under basal conditions in 4 subjects with Graves' disease, using the foreign gas method as modified by Gladstone.[5] To allow for the subnormal vital capacity, the volumes of the gases inhaled were reduced and instead of attempting to wash out the dead space, which becomes difficult when the vital capacity is reduced, 2 mixing breaths into the front rebreathing bag were taken before drawing the first sample, and 2 more breaths before drawing the second sample. Each sample was drawn at the end of expiration. Each respiratory cycle took 2.5 seconds (the breathing and sampling being guided by the spoken directions of an accurately timed phonograph record) so that the test was completed in 10 seconds. The bag contents were as follows:

Side Bag		Front Bag
400 cc.	C_2H_2	120 cc.
	CO_2	55
100	O_2	
500	air	825

The findings are summarized in Table I. To check the adequacy of the mixing procedure an additional sample was drawn simultaneously with the first alveolar sample but from the distal end of the front rebreathing bag. The A-V difference calculated from the additional sample paired with the conventional second sample will show a deviation determined by the errors due to gas analysis and possible inadequate mixing. The sum of the last 2 errors, however, does not exceed that due to gas analysis alone. One may assume, therefore, that the mixing is adequate.

Previous studies[6] have indicated that recirculation of the foreign gas in appreciable amounts occurs at 10 seconds or promptly thereafter in the rebreathing procedure described. Since such recirculation would increase the calculated A-V difference an additional sample was drawn at the end of the fifth breath, *i. e.*, after 12.5 seconds of rebreathing, and that sample paired with the conventional first sample. The values thus obtained are erroneously high due to the large error of recirculation.

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[5] Gladstone, S. A., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1321.

[6] Gladstone, S. A., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1319.

TABLE I.
Metabolic and Circulatory Functions in Graves' Disease.

Subject	Age, yr.	Sex	Weight, lb.	Height, inch.	Pulse Rate	Blood Pressure		Oxygen Consumption, cc. per min.	Basal Metabolic Rate	A-V Difference	Cardiac Output (liters per min.)	Systolic Output, cc. per beat	Pulse Pressure, mm. of Hg.
						syst.	diast.						
W.M.	31	M	110	69	78	115	55	268	+22	35.4	7.6	97	60
M.O.	22	F	100	61	68	110	60	240	+35	38.2	6.3	71	50
D.O.	25	F	102	63	98	95	48	214	+16	23.9	8.9	91	47
J.P.	33	M	129	68	102	130	70	365	+60	38.3	9.5	93	60
Aver. Graves'					92	113	58		+33	34.0	8.1	88	54
Aver. normal[7]					59	107	75			53.6	4.2	70	32
% change					+56	+6	—23		+33	—37	+93	+26	+69

TABLE II.
To Demonstrate Adequacy of Mixture (B) and Effect of Undue Prolongation (C) on the A-V Difference Obtained by the Method Described (A).

	A	B	C
	35.4	35.2	
	38.2	41.7	48.7
	23.9	27.5	43.5
	38.3		49.1
Average	34.0	35.1	47.1

Column A indicates the values for the A-V difference obtained by the method described in 4 cases of Graves' disease. Column B indicates the values obtained when the first sample is drawn from the distal end of the bag. Column C indicates the values obtained when the last sample is drawn at 12.5 seconds.

The agreement in columns A and B indicates the adequacy of the mixing procedure. The increase in column C demonstrates the effect of the error due to recirculation of the foreign gas when the rebreathing procedure is unduly prolonged.

To estimate the nature and extent of the deviation from normal in the several functions studied, the average results may be compared with the averages obtained in 6 nomal young men who have been studied under the same conditions, *i. e.*, fasting, at rest, recumbent in a deck chair for 2 hours.[7] The hyperactivity of the circulation in Graves' disease becomes even more striking when one considers that the subjects of the normal group weighed about 40% more than the hyperthyroid patients that were the subjects of the present study. In the 4 cases of Graves' disease studied on the average the B.M.R. is increased by 33%, the A-V difference is decreased by 37%, the cardiac output is increased by 93%.

It is apparent that the cardiac output and systolic output are indirectly calculated from the A-V difference, the accuracy of which will determine the reliability of the quantitative functions calculated from it. In Graves' disease as compared with normal subjects, Fullerton and Harrop[3] as well as Rynearson and Boothby[4] reported no significant change in the A-V difference. Liljestrand and Stenström[1] reported a decrease of about 20%. Bansi[2] reported a decrease of 33%,[8] *i. e.,* 55 cc. for the normal A-V difference, 37 cc. in Graves' disease. The present findings are in close agreement with those of Bansi, being 53.6 for the normal A-V difference and 34 cc. in Graves' disease, representing a decrease of 37%. Rynearson and Boothby have discussed this discrepancy and have explained it as a possible effect of iodine therapy in their patients. Since the first 3 of our patients had received iodine, the consistent finding of a low A-V difference would tend to invalidate that explanation. Recent studies[9] of the foreign gas method have indicated that after 10 seconds of rebreathing of a foreign gas under the conditions of the test, appreciable amounts of gas return to the lungs in the recirculated blood and retard the diffusion of the gas from the lungs into the blood. When the rebreathing extends beyond 20 seconds as in the Marshall-Grollman procedure used by Fullerton and Harrop as well as by Rynearson and Boothby, the errors become large enough to render the method insensitive and unreliable. The procedure of Krogh and Lindhard as used by Liljestrand and Stenström is definitely shorter in duration (about 15 seconds) and includes a period of 10 seconds during which the breath is held. These considerations, as well as the finding by Liljestrand and Stenström that the A-V difference is somewhat decreased in Graves' disease, suggest that the error due to recirculation of the foreign gas is possibly present but is not nearly so great as in the Marshall-Grollman procedure.

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[7] Gladstone, S. A., *Am. J. Physiol.*, 1935, **112**, 705.

In addition to subserving the metabolic needs of the tissues, the blood also acts as part of the cooling system for dissipating heat, especially through the skin capillaries. This purely physical function of the blood does not involve a loss of oxygen. The venous blood coming from the dilated capillaries of a hot skin will have a high oxygen content, will tend to increase the oxygen content of the mixed venous blood and will accordingly decrease the A-V difference, as has been found in these patients with Graves' disease.

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[8] Grollman, A., The Cardiac Output of Man in Health and Disease. Baltimore, 1932.

[9] Gladstone, S. A., Cardiac Output and Arterial Hypertension. New York, 1935.

Since the precise nature of the underlying metabolic disturbance of Graves' disease is as yet unknown, the relation between it and our present findings is undetermined. It has been found that hyperthyroid patients require more oxygen to perform a given physical task than normal subjects.[10] These findings as well as our own suggest that in Graves' disease a disproportionate amount of the energy available to the organism is dissipated as heat, and that Graves' disease may not be looked upon merely as a quickening to the same relative extent of all metabolic functions, but represents a different level of dynamic equilibrium, with an excess of certain exothermic catabolic oxidations, or a relative decrease in certain endothermic anabolic changes.

Summary. In 4 cases of Graves' disease with an average basal metabolic rate of +33%, the arterio-venous oxygen difference was found to be decreased by 37%. The average cardiac output was 8.1 liters per minute compared to a normal of 4.2, representing an increase of 93%. The relation of the present findings to those previously reported, to the question of method of cardiac output determination, and to the possible nature of the underlying metabolic disturbance in Graves' disease is briefly discussed.

8711 P

Barbiturate-Coriamyrtin Antagonism.

A. H. MALONEY. (Introduced by G. B. Roth.)

From the Department of Pharmacology, Howard University School of Medicine, Washington, D. C.

At the time when we established the antidotal action of picrotoxin on the barbiturates[1] we indicated our intention of investigating the action of other picrotoxin-like substances. This paper reports the antidotal action of coriamyrtin in rabbits subjected to lethal doses of amytal, pernoston and nembutal.

The same method of procedure was followed in this work as in our early investigations of picrotoxin.[1, 2] The animals were given single lethal doses of the barbiturate in increasing increments up to the limit of effective therapeusis and as soon as narcosis occurred coriamyrtin was administered in fractional doses at intervals P.R.N.

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[10] Boothby, W. M., and Sandiford, S., *J. Am. Med. Assn.*, 1923, **81**, 795.

[1] Maloney, A. H., Fitch, R. H., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1931, **41**, 465.

[2] Maloney, A. H., and Tatum, A. L., *J. Pharm. and Exp. Therap.*, 1932, **44**, 337.

The initial dose of coriamyrtin was so graded as to elicit a mild state of reflex stimulation, evidenced by an increased rate of respiration and a manifestation of such gross reflexes as the whisker twitch, winking and (or) nystagmus, jerking movements of the limbs and ears, and coarse body tremors. Great care was taken in the lower barbituratized groups to grade the initial dose of coriamyrtin so as to avoid throwing the animal into violent convulsions. Mild seizures in such cases are inconsequential and pass off within a few minutes. When the respiration rate became slow again and the gross reflexes disappeared, to determine the degree of depression pinching the tip of the tail or ear was done. This served as an index for the quantitative estimation of the succeeding dose of coriamyrtin. Our technique was to try to maintain such a degree of sensitivity as to elicit a reaction of resistance to this mechanical mode of stimulation at all times. This procedure was repeated until the effects of the barbiturate were palpably beginning to wear off, as evidenced by the persistence of the induced hypersensitivity. The barbiturates were administered intraperitoneally as a 5% aqueous solution while the coriamyrtin was injected intravenously as a 0.1% solution.

Preliminary experiments to determine the pharmacodynamic activity of our sample of coriamyrtin showed that the glucoside has a minimal convulsive potency of 0.14 mg./kg., and a minimal lethal potency of 0.40 mg./kg., both intravenously, in the rabbit. In most instances 75% of the normal lethal intravenous dose was adequate to produce initial stimulation in the animals subjected to the lower ranges of the barbiturate dosages. However in the higher levels we have frequently had to give 1.5-2 mg./kg. as a single first dose to initiate the desired stimulatory effects.

With amytal sodium 100% recoveries were obtained up to a little more than 1.5 beyond the M.L.D. (150 mg./kg.). At twice the M.L.D. 33.3% recovered. This dosage bordered on the upper limit of effective therapeusis. The antidotal ratio of coriamyrtin and amytal for the total number that recovered was 1:43.4

Coriamyrtin was 100% effective against 1 and 1.3 times the M.L.D of nembutal. At 1.7 times the M.L.D. its efficiency was 80%. Beyond this level, however, we were unable to save any animal, 3 trials each having been made with doses of 125 mg./kg.

(1.9×M.L.D.), 150 mg./kg. (2.4×M.L.D.), and 200 mg./kg. (3.1×M.L.D.). The antidotal ratio of coriamyrtin and nembutal for the total number of survivals was 1:20.55.

In the pernoston series 70% recovered at a dosage slightly above the M.L.D. (1.2) and 60% recovered at a dosage 2.1 times the M.L.D. This dosage level represented the upper limit of effective therapeusis. The results in this series corroborate our previous observations[3] of the wide range of therapeutability of pernoston as compared with the non-halogenated barbiturates. The antidotal ratio of coriamyrtin and pernoston for the total number of survivals was 1:34.6.

The action of coriamyrtin is as quick as that of picrotoxin but is of much shorter duration. This necessitates constant surveillance of the severely poisoned animal and the prompt administration at rather frequent intervals of small fractional doses of coriamyrtin as soon as indicated. Further detailed studies are in progress on coriamyrtin and tutin.

We wish to express appreciation to the Eli Lilly Research Laboratories for supplying amytal and coriamyrtin, the Abbott Laboratories for nembutal and the Riedel de Haen Company for pernoston used in this study.

8712 P

Poliomyelitis Histology in Rhesus Monkeys: Virus Introduced Via Gastrointestinal Tract.*

JOHN A. TOOMEY.

From the Department of Pediatrics, Western Reserve University, and the Division of Contagious Diseases, City Hospital, Cleveland, O.

When poliomyelitic virus was injected intracerebrally into *M. rhesus* monkeys no histologic evidence of its presence was observed in sections of tissues from the central nervous system until at least 3 days (usually 4 days) had elapsed following the injection. This corresponded to the time when symptoms of the disease began to appear.[1] When virus was injected directly into the median nerves of 6 animals with or without irritation of the nerves, no histologic evidence of its presence was found on the proximal side a scant inch beyond the injection. When Fairbrother and Hurst[1] injected virus into the sciatic nerve, there was a local reaction, but the nerves were negative a short distance beyond the injection. This observation was confirmed by us in 3 animals. Thus, there is an interval elapsing between the injection of virus and the onset of symptoms of the disease. It is clear that the approximate interval after injection before symptoms appear and the length of time before there is histologic evidence of the disease probably coincide.

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[3] Maloney, A. H., *J. Pharm. and Exp. Therap.*, 1933, **49**, 133.

* Aided in part by a grant received from The President's Birthday Ball Commission for Infantile Paralysis Research.

[1] Fairbrother, R. W., and Hurst, E. W., *J. Path. and Bact.*, 1930, **33**, 17.

During the summer of 1935 44 animals were injected subserosally in the manner previously described.[2] Sections of the central nervous system were cut, stained in various ways and studied. It was found that, after the introduction of the virus by way of the gastrointestinal tract, histopathologic evidence of disease was regularly found in the lumbar area of the cord within 24 hours after injection. The first reaction seen was destruction of neurons in the cord. Marked increase in microglial elements (Wickman's Polyblasts) followed so soon after this reaction that it seemed to be almost coincidental. In from 3 to 4 days increase and dilatation of capillaries began. In many animals, neuronophagic plaques surrounded practically all of the cells in a field, and, in others, this change was accompanied by the usual perivascular cuffing and round cell infiltration, which never occurred prior to the 5th day after injection.

8713 P

Significance of Carbohydrate Metabolism and Local Acidosis in Inflammation.*

VALY MENKIN AND CHARLOTTE R. WARNER.

From the Department of Pathology, Harvard University Medical School, Boston.

Earlier studies by one of the writers have indicated that the cytological sequence in inflammation, characterized by primary

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[2] Toomey, John A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1015.

* Read before the American Association of Pathologists and Bacteriologists, April 9, 1936.

Aided by grants from the Wellington Research Memorial Fund, the Permanent Charity Fund of the Harvard Medical School, and the Committee on Scientific Research, American Medical Association.

TABLE I.

The Relationship between Glycolysis and the Cytological Picture in Acute Inflammation.

Dog No.	Amount of turpentine injected	Total duration of inflammation hrs.:mins.	Sugar mg./100 cc.		Lactic Acid mg./100 cc.		pH		Cytology of Exudate	
			Blood	Exudate	Blood	Exudate	Blood	Exudate	% of polymorphonuclears	% of mononuclear phagocytes
9-2	1.5 cc.	0:0	100.0	—	—	—	7.5	—	—	—
		19:10	88.1	96.0	10.8	54.6	7.45	7.43	Relatively	acellular
		47:10	111.9	57.9	13.0	27.0	7.48	7.33	85	15
	2.0 cc. (reinjected on third day)	68:00	145.5	116.8	16.0	27.8	7.48	7.33	74	26
		96:15	81.2	13.9	21.9	135.8	7.43	6.5	15	85
		115:00	138.6	6.4	38.4	248.6	7.43	6.5	2	98
		167:00	138.6	34.7	14.7	217.1	7.45	6.5	2	98

infiltration of polymorphonuclear leucocytes followed by mononuclear phagocytes, seems to be conditioned by the local hydrogen-ion concentration of the exudate.[1] With the developing local acidosis in an area of inflammation polymorphonuclear cells degenerate and are replaced by macrophages. If the pH is maintained above 7.0 the polymorphonuclear cells predominate. The cytological picture can be fairly well predicted from the pH of the exudate and vice-versa. In many cases the pH changes appear to precede the cytological shift which, however, invariably follows. Further studies show that the mechanism of local acidosis in inflammation is referable to a disturbance of local carbohydrate metabolism. With impairment in the local circulation, as indicated by lymphatic blockade and thrombosis of small vessels, it is conceivable that a state of relative anoxemia develops.[2] Anaerobic glycolysis follows. Lactic acid is found to increase two to fourfold as the inflammation progresses. The sugar in the exudate correspondingly falls sharply, revealing a conversion into lactic acid. Most of the inflammatory exudates studied were induced by the injection of small amounts of turpentine, administered sometimes in divided doses, into the pleural cavity of dogs. With the progress of the inflammatory reaction the local alkali reserve soon becomes depleted, as determined by the volume percent of CO_2 content. An uncompensated acidosis follows; the volume percent of CO_2 falling in some instances from 40 to 29 and even to lower levels. The pH falls and the polymorphonuclear cells are replaced by macrophages. Below a pH of 6.8 or 6.7 most types of leucocytes are damaged and frank pus results. The observations indicate that the cytological picture in inflammation seems to be conditioned by the pH, which in turn is referable to the increased rate of glycolysis and the local depletion of the alkali reserve. These studies have obvious implications in regard to the factors determining various histological manifestations of inflammatory lesions. Their bearing on the mechanism of suppuration is likewise evident and is being studied in the case of lesions induced by various pyogenic and non-pyogenic organisms. The present conclusions are based on results obtained with 7 dogs. For the purpose of illustration a type experiment is presented in Table I. The details of this investigation will form the subject of a separate communication.

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[1] Menkin, V., *Am. J. Path.*, 1934, **10**, 193.

[2] Menkin, V., *Arch. Path.*, 1931, **12**, 802.

8714 P

Effect of Certain Sulfur Compounds on Coagulation of Blood.

J. H. Sterner and Grace Medes.

From the Medical Service of the Lankenau Hospital and the Lankenau Hospital Research Institute.

The initial observation of Mueller and Sturgis[1] that cysteine inhibits the coagulation of whole blood *in vitro* has since been confirmed and elaborated by Carr and Foote,[2] who suggested that the delay in coagulation might be due to certain changes which they observed in the physical state of the fibrinogen. Kühnau and Morgenstern,[3] working with glutathione, also described inhibition of coagulation within a certain range of pH. Since the mechanism of the reaction has not been explained, the experiments reported here have been undertaken. Three lines of investigation have been carried out:

1. *In vivo experiments in human subjects.* Cysteine and methionine, ingested and injected intravenously in amounts varying from 1 to 3.5 gm., prolonged the bleeding time as indicated by the Ivy method from a 4 minute upper limit of normal to 15 minutes (methionine, 1.31 gm. intravenously) and delayed the coagulation time as shown by the 8 mm. tube method, from an 8 minute upper normal to 16 minutes (cysteine, 1 gm. intravenously and methionine, 1.31 gm. intravenously).

2. *In vitro experiments with whole blood.* Cysteine hydrochloride, taurocholic acid, taurine, methionine, glycine, alanine and cysteic acid, neutralized to pH 7, were added in graded amounts giving final concentration of from 4.4×10^{-5} *M* to 0.18 *M*, and the coagulation time was determined by the 8 mm. tube method. Glycine, alanine and cysteic acid did not retard coagulation. Methionine, in contrast to its behavior *in vivo,* also had no effect in delaying coagulation. Cysteine produced a significant effect at a 0.046 *M* concentration, with a coagulation time of 18 minutes. At 0.18 *M* concentration, the coagulation time was 53 minutes. Taurine showed a definite effect at 0.023 *M* concentration (16 minutes), with a 90 minute coagulation time at 0.18 *M* concentration. Taurocholic acid produced a significant effect at 0.011 *M* concentration (34 minutes),

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[1] Mueller, J. H., and Sturgis, S., *Science,* 1932, **75**, 140.

[2] Carr, J. L., and Foote, F. S., *Arch. Surg.,* 1934, **29**, 277.

[3] Kühnau, J., and Morgenstern, V., *Z. physiol. Chem.,* 1934, **227**, 145.

with no coagulation during 24 hours in concentrations of 0.023 to 0.18 *M*.

3. *In vitro experiments with isolated components of the blood clotting system.* The components of the coagulation system were isolated by the following methods: Prothrombin was prepared from horse, rabbit and human plasmas by the method of Mellanby.[4] Fibrinogen was repeatedly salted out from horses' plasma with sodium chloride (Eagle[5]). The tissue factor was obtained from 2 sources, (a) platelet suspension (Eagle[5]), and (b) desiccated rabbit lung (Eagle[6]), by drying in the Flosdorf-Mudd desiccator.* Calcium was added as 1% $CaCl_2$. The pH was controlled at 7.0 ± 0.1 with brom thymol blue as indicator. From a stock solution of cysteine hydrochloride, freshly prepared immediately before using, and adjusted to a pH of 7.0 ± 0.1, a series of dilutions was made. In a typical experiment the following amounts of the various constituents were used: prothrombin solution, 0.8 cc.; $CaCl_2$ solution, 0.01 cc.; platelet suspension, 0.02 cc. or approximately 2 mg. of desiccated rabbit lung; fibrinogen solution, 0.8 cc. When cysteine was added to the system, 0.4 cc. were used and 0.4 cc. of physiological salt solution added to the controls.

Cysteine, added to the coagulation system before the formation of thrombin had an inhibiting effect similar to that in the whole blood experiments. This inhibiting effect was constant regardless of the length of time allowed for the formation of thrombin. When thrombin, formed by the interaction of prothrombin, tissue factor and calcium, was subjected to cysteine in the higher concentrations given above, only a slight inhibiting effect was produced.

Each of the elements was then subjected separately to the action of cysteine and subsequently freed from traces of cysteine by suitable methods. No effect on the rate of coagulation was produced by the action of cysteine on (a) fibrinogen, (b) platelet suspension, or (c) platelet suspension plus calcium. When prothrombin was acted upon by cysteine in 0.4 *M* concentration, the coagulation time was 540 minutes, in 0.2 *M* concentration the coagulation time was 28 minutes and in 0.1 *M* concentration, 5 minutes and 5 seconds. In the control tube coagulation occurred in one minute and 20 seconds. When sodium hypophosphite was employed in place of cysteine in equimolar concentrations, no inhibition of the coagulation was detected.

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[4] Mellanby, John, *Proc. Roy. Soc. Lond.*, 1931, B, **107**, 271.

[5] Eagle, Harry, *J. Gen. Physiol.*, 1934-5, **18**, 531.

[6] Eagle, Harry, personal communication.

* The authors are indebted to Dr. Flosdorf of the University of Pennsylvania for use of their desiccator and to Sharpe and Dohme for horse blood.

8715 P

Hypophysis-Thyroid-Gonad Relationship.*

SAMUEL L. LEONARD.

From the Biology Department, Union College, Schenectady, N. Y.

Thyroidectomy in male and female rats will increase the effectiveness of crude hypophyseal gonad stimulating extracts but the removal of this gland will not increase the effectiveness of pregnancy urine or pregnancy mare serum extracts (Smith,[1] Schockaert,[2] Fluhmann,[3] Leonard and Hansen[4]). Since Fluhmann[3] showed that thyroid feeding in rats decreased the effectiveness of both types of gonad stimulating hormones (hypophyseal and pregnancy urine), he suggested that the thyrotropic principle, present in the crude hypophyseal extracts, induced a hyperthyroid condition in the normal rats, which made the gonad stimulating hormone less effective. Pregnancy urine, not possessing a thyrotropic factor, was equally effective in normal and operated animals. This report will show that follicle stimulating hormone from hypophyseal or urinary sources and free of the thyrotropic hormone is more effective in the absence of the thyroid.

Immature female rats 24 to 26 days of age were thyroidectomized and injected with extracts of the urine of castrate women and of a man with a testis tumor, for 8 to 9 days. A similar number of control rats received only injections of the hormone. Another group of operated and control rats were treated with hypophyseal follicle stimulating hormone, prepared by Drs. Fevold and Hisaw,† for 5 to 6 days. None of the extracts were found to contain a thyrotropic factor when tested on young guinea pigs or hypophysectomized rats in the doses given. The ovaries and uteri were weighed at autopsy. More than 50 rats were used in these

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* Aided by grants from the Rockefeller Foundation.

[1] Smith, *Sex and Internal Secretions*, 1932, Ed. by Allen.

[2] Schockaert, *Compt. Rend. Soc. Biol.*, 1931, **108**, 431.

[3] Fluhmann, *Am. J. Physiol.*, 1934, **108**, 498.

[4] Leonard and Hansen, *Anat. Rec.*, 1936, **64**, 203.

† Acknowledgment is made to Doctors Fevold and Hisaw for their kindness in preparing the pituitary follicle stimulating hormone.

experiments and only representative experiments are given in Table I.

TABLE I.
Effect of Thyroidectomy on the Action of Follicle Stimulating Hormone.

No. of Rats	No. of Days	Amt. of Hormone	Av. Ovarian Wt., mg.		Av. Uterine Wt., mg.		% difference	
			Exp.	Cont.	Exp.	Cont.	Ovary	Uterus
13	6	F.S.H. .33 cc.	42.0	30.4	141.5	90.8	38.1	55.8
5	9	Testis tumor equiv. of 100 cc. urine	49.0	18.8	153.6	65.5	160.6	134.5
5	8	Spayed woman equiv. of 100 cc. urine	39.0	25.6	121.0	42.4	52.3	185.3

From the results in Table I it is seen that the follicle stimulating hormone prepared from the hypophysis and urine and free from the thyrotropic hormone is more effective in the absence of the thyroid. The ovaries are from 38.1% to 160.6% heavier and contain only follicles. The larger ovaries in the thyroidectomized rats produce greater amounts of female sex hormone as the uteri of the thyroidectomized animals are from 55.8% to 185.3% heavier than those of the control.

Since pregnancy urine was previously shown to be equally effective in normal and thyroidectomized rats, it is concluded that the presence of the thyroid gland inhibits or prevents, to a certain degree, the action of the follicle stimulating hormone but does not prevent the action of pregnancy urine hormone (a luteinizer). Thus, the thyroid gland has a differential effect in inhibiting two types of sex stimulating hormones, a follicle stimulator from the hypophysis and urine and a luteinizer from urine.

It has been previously shown that the addition of thyrotropic hormone to pregnancy urine will not inhibit the effect of pregnancy urine (Leonard, Hisaw and Fevold[5]). It would seem from this fact that the increased effectiveness of a crude hypophyseal extract, containing both thyrotropic and follicle stimulating hormone, in the thyroid-less rat, is due, at least in part, to the enhanced effectiveness of the follicle stimulating hormone present.

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[5] Leonard, Hisaw and Fevold, PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 319.

8716 C

Effect of Local Ischemia upon Human Nerve Fibers *in vivo.*[*]

I. MACLAREN THOMPSON AND H. STEWART KIMBALL.[†] (Introduced by David M. Greenberg.)

From the Division of Anatomy, University of California Medical School, Berkeley.

In each experiment ischemia was induced in the forearm and hand by inflating to a pressure of about 160 mm. Hg. a Riva-Rocci sphygmomanometer applied above the elbow. The median, ulnar or radial nerve was stimulated at the wrist by an alternating current of 160 cycles per second, using the generator and electrode described by Thompson *et al.*[1]; the minimal current (expressed as ma × v) evoking sensation was recorded as an indicator of the threshold for stimulation of sensory fibers. A threshold reading was taken every 2 minutes after inflation of the sphygmomanometer, for about 16 minutes.

Thirteen such experiments were performed, involving 4 subjects. The results are exemplified in the following experiment, wherein the right radial nerve was stimulated; the first figure of each pair denotes the number of minutes after the induction of ischemia, the second figure is the threshold at that time: 0, 5.5; 2, 7.2; 4, 5.2; 6, 2.8; 8, 2.4; 10, 1.1; 12, 0.8; 14, 1.6; 16, 11.5. In every experiment there was, on the whole, a rapid and progressive lowering of the threshold for about the first 12 minutes. The average fall in threshold per unit of time significantly exceeded the corresponding value in each of 4 groups of control experiments; the most important group consisted of 14 experiments wherein nerves were similarly stimulated *proximal* to a sphygmomanometer arresting the circulation. In 5 of the 13 instances the threshold drop progressed throughout the duration of the experiment; but on 8 occasions (though in no control experiment) there was a terminal rise (exemplified above) to values equalling or exceeding the initial figure. We infer that the ischemia of the nerve first increased and later diminished the irritability of those fibers first stimulated by a current increasing from zero.

These results confirm the opinion of Lewis, Pickering and Roths-

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[*] Aided by a grant from the Board of Research of the University of California.

[†] Mr. Kimball's share in this work was incorporated into his M.A. thesis, deposited in the Library of the University of California.

[1] Thompson, I. M., *et al., Univ. Calif. Publ. Anat.*, 1934, **1**, 167.

[2] Lewis, T., Pickering, G. W., and Rothschild, P., *Heart*, 1931, **16**, 1.

child[2] that such ischemia affects the nerve fibers, not merely the nerve endings. But Bishop, Heinbecker and O'Leary[3] attribute the effect to the pressure whereby the ischemia is produced. In view of the work of Erlanger, Gasser, and others, it seems clear that we studied the thresholds of large, easily-stimulated fibers. The initial drop in threshold, followed by a rise above the normal, so closely parallels the observations of Heinbecker[4] on threshold changes in anoxemic excised frog nerves that we consider our results to confirm Heinbecker's work and extend it to human nerves *in vivo*.‡ But asphyxia attacks the small fibers first,[4] whereas pressure affects first the large fibers (Gasser and Erlanger[5]); in our experiments the large fibers, though more susceptible to pressure, manifested behavior characteristic of asphyxia. Our work thus presents another aspect of a difficulty that has confronted previous investigators, and attempts to explain which have been offered by Zotterman,[6] by Bishop, Heinbecker and O'Leary[3] and by Gasser.[7] Both pressure and anoxemia seem to contribute to the result. (Terms like anoxemia and asphyxia are here used to designate the total chemical environment of the nerve fibers determined by circulatory arrest; a more particular discussion is offered by Gerard.[8]) It may be that pressure increases the susceptibility of the fibers to ischemia; this would explain the early onset of anoxemic phenomena in the large fibers. But how could pressure applied in the arm affect susceptibility to asphyxia throughout the forearm and hand? Possibly by blocking the passage along the nerve fibers of those "trophic" influences from the nerve cells whose existence is suggested by Wallerian degeneration, and has recently been upheld on other grounds by Parker.[9] §

Alternatively, and perhaps preferably, accepting the view that pressure does not contribute toward the phenomenon,[2, 7] our results may be regarded as confirming, from a study of irritability, Gasser's[7] conclusion from experiments on conductivity, that in mammalian nerves *in vivo* asphyxia affects the large fibers first.

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[3] Bishop, G. H., Heinbecker, P., and O'Leary, J., *Am. J. Physiol.*, 1933, **106**, 647.

[4] Heinbecker, P., *Am. J. Physiol.*, 1929, **89**, 58.

‡ Comparison of our data with the time-relationships recorded by Heinbecker[4] leads us to believe that had the 5 experiments wherein the threshold did not rise been prolonged, that phenomenon would have occurred.

[5] Gasser, H. S., and Erlanger, J., *Am. J. Physiol.*, 1929, **88**, 581.

[6] Zotterman, Y., *Acta Med. Scand.*, 1933, **80**, 185.

[7] Gasser, H. S., *Proc. Assn. Res. Nerv. and Ment. Dis.*, 1935, **15**, 35.

[8] Gerard, R. W., *Am. J. Physiol.*, 1930, **92**, 498.

[9] Parker, G. H., *Am. Nat.*, 1932, **66**, 147.

§ It must be assumed that "trophic" impulses are more easily blocked by pressure than are ordinary nerve impulses.

8717 C

Effect of Lactogenic Hormone on Embryonic Tissues Cultivated *in vitro.*

A. J. SALLE AND I. L. SHECHMEISTER.

From the Department of Bacteriology, University of California, Berkeley.

Since lactogenic hormone* is known to produce a specific effect on epithelial cells of the pigeon crop[1] *in vivo,* experiments were designed to determine whether it would stimulate the growth of embryonic pigeon crop epithelium cultivated *in vitro.* If stimulation could be observed in this manner such a test might be more delicate and superior to the present methods employed for determining hormonal potency.

Preliminary to testing the hormone on the specific crop epithelium, which reacts to it *in vivo,* experiments were carried out on the following non-specific tissues cultivated *in vitro:* (1) Embryonic chick connective tissue, (2) embryonic chick epidermis, (3) embryonic pigeon oesophageal epithelium. Finally the hormone was tested on cultures of embryonic epithelium taken from the crop sac areas known to be reactive *in vivo* in the hatched pigeons.

In all cases the dilutions were performed in triplicate. Also, every experiment was repeated 3 times before the results were reported. Each flask contained about 12 fragments of tissue.

(1) *Embryonic Chick Connective Tissue.* Chick heart tissue from 10- to 14-day-old embryos was used as the source of fibroblasts. The tissues were finely cut into fragments of about 1 mm. in diameter and immersed in Tyrode solution until ready for use.

The embryonic fluid was prepared by mincing 14-day-old chick embryos in a tissue grinder and suspending the pulp in 4 parts of

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* Anterior pituitary mammotropic principle, prolactin, galactin, mammotropin. The preparations used in these experiments were kindly supplied by Dr. W. R. Lyons of the Division of Anatomy. The hormone caused crop sac epithelial growth in 24-48 hours when given in doses as low as 0.1 microgram intradermally over the crop sac area.

[1] Riddle, O., Bates, R. W., and Dykeshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

Tyrode solution. The suspension was centrifugated at high speed to throw down all cellular material after which the clear supernatant fluid was carefully removed with a pipette. This clear liquid was used for coagulating the plasma as well as a nutrient for the tissue cells.

Guinea pig plasma was used in all experiments. This was collected by first drawing 1.0 cc. of a 1-1,000 solution of heparin into a 10.0 cc. syringe and then heart blood to the 10.0 cc. mark. The mixture was quickly centrifugated, the clear plasma removed with a pipette and kept in ice water until ready for use.

The hormone preparation† was added to the solid phase only. The various components and their proportions are summarized in Table I.

TABLE I.

Components	No. of drops used					
	Hormone-Treated Flasks					Control Flasks
Plasma	3	3	3	3	3	3
Tyrode Solution	4	3	2	1	0	5
Embryonic fluid	2	2	2	2	2	2
Hormone Solution	1	2	3	4	5	0
Total No. of drops	10	10	10	10	10	10

The various mixtures were prepared in Carrel flasks. Ten drops of embryonic fluid were added after coagulation of the plasma. This furnished the nutrients for the tissue cells.

The growth in all flasks, both hormone-treated and non-hormone treated was of the same order after an incubation period of 48 hours.

(2) *Embryonic Chick Epidermis.* Skin tissue from 7, 9, 10, and 12-day-old chick embryos was used as the source of epithelial cells. Mixed growth of fibroblasts and epithelial cells occurred in many of the fragments but the fibroblastic growth was disregarded.

In the first series of experiments graduated amounts of hormone was added to the embryonic fluid following coagulation of the plasma. No hormone was present in the solid phase. Epithelial stimulation was negative after an incubation period of 72 hours.

In the following series of experiments the hormone was added to the solid phase only. The set-up is given in Table II.

Ten drops of embryonic fluid was added following coagulation of the plasma.

In another series of experiments the same set-up as given above

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† The hormone preparation contained 1 gm. of organic solids in 100 cc. of solution. This is, for convenience, called a 1% solution.

TABLE II.

Components	No. of drops used				
	Hormone-Treated Flasks				Control Flasks
Plasma	3	3	3	3	3
Tyrode Solution	3	2	1	0	5
Embryonic Fluid	4	4	4	4	4
Hormone Solution	2	3	4	5	0
Total No. of drops	12	12	12	12	12

was employed except that hormone was added to both the solid and liquid phases. Consistently negative stimulation was obtained in all experiments.

(3) *Embryonic Pigeon Oesophageal Epithelium.* In the experiments to follow embryonic pigeon oesophageal epithelium was employed. The oesophagus in the region of the crop sac was removed aseptically from a pigeon just before hatching. The components of the cultures and their proportions were the same as given in Table II.

In the first series of experiments hormone was added to the solid phase only. In the second series hormone was added to the liquid phase only. Tyrode solution was substituted for the hormone in the solid phase. In the final series hormone was added to both solid and liquid phases. Growth stimulation was negative in all experiments after an incubation period of 48 hours.

(4) *Embryonic Pigeon Crop Epithelium.* Tissue from the embryonic crop gland of a pigeon was used. The glandular tissue was removed aseptically and reduced to fragments as uniform in size as possible.

The components and proportions of each were the same as given in Table II. The following series of experiments were performed:

1. Tissue fragments bathed in Tyrode solution previous to embedding. Hormone solution was added to (a) liquid phase only, (b) solid phase only, and (c) both solid and liquid phases.

2. Tissue fragments bathed in hormone solution previous to embedding. Hormone solution added to (a) liquid phase only, (b) solid phase only, and (c) both liquid and solid phases.

(3). Tissue fragments were embedded and allowed to incubate for 36 hours before adding hormone to the liquid phase. Five dilutions of hormone were used ranging from 1:10 to 1:100,000.

Measurements of the areas of the tissue fragments were made and percentage increases recorded. A total of 220 measurements on 93 tissue fragments were made. The figures showed no growth stimulation of the fragments in the presence of hormone.

It may be concluded that lactogenic hormone, although capable of producing a specific effect on epithelial cells of the immature pigeon crop *in vivo,* is unable to stimulate embryonic crop tissue when cultivated *in vitro.* Non-specific tissues such as embryonic chick connective tissue, embryonic chick epidermis and embryonic pigeon oesophageal epithelium also failed to show growth stimulation in the presence of the hormone.

8718 P

Effect of Autoclaved Pancreas upon Lipids of Blood and Liver in Depancreatized Dogs Maintained with Insulin.*

A. KAPLAN AND I. L. CHAIKOFF.

From the Division of Physiology, University of California Medical School, Berkeley.

It was first shown in this laboratory that raw pancreas contains a factor (or factors) active on the blood lipids of the completely depancreatized dog maintained with insulin.[1] When the glandular tissue was added to the diet immediately after pancreatectomy, a rise above normal in blood lipid constituents occurred. The removal of the glandular tissue from the diet resulted in an abrupt fall in the lipid constituents, in particular, cholesterol esters. The essential effect of pancreas feeding upon blood lipids consisted in the maintenance of a level, particularly cholesterol, above the preoperative or normal; this effect was produced irrespective of whether the concentration of the blood lipids was normal or subnormal at the time when feeding of the glandular tissue was instituted.

It has already been shown that the blood lipid factor is not choline.[2] In a further study of the mechanism whereby raw pancreas produces these lipid changes, it seemed of interest to investigate the effects of pancreas that had been autoclaved for 30 minutes at 20 lb. pressure. Such a procedure destroys the enzymes but leaves unaltered the caloric value of the ingested pancreas.

A typical result of the effect of autoclaved pancreas on the blood

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* Aided by a grant from the Research Board of the University of California. The insulin used in this investigation was generously supplied by Eli Lilly and Company.

[1] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1935, **112**, 155.

[2] Chaikoff, I. L., and Kaplan, A., PROC. SOC. EXP. BIOL. AND MED., in press.

cholesterol is shown in Table I. For the first 37 days following pancreatectomy Dog D-73 received 125 gm. of raw pancreas twice daily in addition to the regular stock diet. Determinations made on 2 occasions showed that the blood lipid concentration was definitely above normal during this period. For the next 19 days, autoclaved pancreas, in equal amounts, was substituted for the raw glandular tissue. A definite fall in both free and esterified cholesterol occurred. In 3 other dogs it was found that when autoclaved pancreas was added to the diet of dogs in which the blood lipid level fell below normal, a rise above the preoperative value did not occur. It may, therefore, be concluded that the factor in the pancreas responsible for the maintenance of a blood lipid level above normal is partially or completely destroyed by autoclaving.

TABLE I.

Effect of Raw and Autoclaved† Pancreas upon Whole Blood Cholesterol of Dog D-73.

Interval since Pancreatectomy days	Duration of raw pancreas in diet‡ days	Duration of autoclaved pancreas in diet‡ days	Cholesterol			
			Total mg. per 100 cc.	Free mg. per 100 cc.	Ester mg. per 100 cc.	Ester % of total
29	29		209	127	82	39
37	37		218	123	95	44
45		8	132	99	33	25
56		19	112	103	9	8

†Autoclaved for 30 min. at 20 lb. pressure.
‡125 gm. twice daily in addition to stock diet.

The accumulation of large amounts of lipids is characteristic of livers of depancreatized dogs receiving no raw pancreas in their diets.[3] Despite its inability to raise the preöperative level of the blood cholesterol, autoclaved pancreas was found effective in preventing the deposition of these excessive amounts of lipids in the livers of 2 dogs. Moreover, in a depancreatized dog in which a fatty liver had been established, the fat content of this organ was reduced to normal by the addition of autoclaved pancreas to the diet. In all 3 animals that had received autoclaved pancreas, total fatty acids ranged from 2.7 to 2.9% of the wet weight of the liver, a range that closely approximates the normal.

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[3] Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1935, **108**, 201.

8719 P

Developmental Mechanics of the Central Nervous System in *Triturus torosus.*

J. Frank Daniel.

From the Department of Zoology, University of California, Berkeley.

By the use of a Rotary Disc[1] the development of the central nervous system may be followed step by step in its natural surroundings within the jelly layers of the egg.

The surface material from which the central nervous system forms occupies a position in the young gastrula well above the blastopore and between it and the animal pole, and at this stage appears to be much like the remaining surface material that gives rise to the epidermis.

As the roof of the digestive tract (the organizing center of Spemann) infolds and moves up into contact with the surface material, the latter is induced to form the medullary plate.

By proper illumination the movement of the organizing center of Spemann, like a curtain within the egg, may be observed as it comes up under the prospective medullary material, and the procedure of shaping out the plate may be followed in some detail.

The first change in the material is its thinning out as the median longitudinal groove about the middle of the plate and just above the chorda; later this groove reaches the infundibular recess anteriorly and the blastopore posteriorly. Next a double row of pigment appears on left and right sides, outlining the lateral boundaries of the medullary plate. Neural folds appear between these double bands of pigment and extend gradually backward and forward. Anteriorly left and right folds approach the midline, where they remain separated for some time.

The relation of the organizing center to the surface material often differs considerably on left and right sides. Contact earlier on one side may result in a well-formed neural fold on that side before pigment bands appear on the other side, and a delay of 8 to 10 hours in the completion of the forward part of the plate has been observed to accompany marked cervical flexure.

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[1] Daniel, J. F., and Burch, A. B., *Univ. Calif. Publ. Zool.,* 1933, **39**, 201.

8720 C

Nucleotide Nitrogen Content of Human White Blood Cells.*

Frank Worthington Allen, Salvatore P. Lucia and John J. Eiler.

From the Divisions of Biochemistry (Berkeley) and Medicine (San Francisco), University of California Medical School.

In a previous communication[1] on the nucleotide content of pathologic human whole blood, it was stated that the correlations between nucleotide nitrogen and the percentage of hemoglobin and the red blood corpuscle count are high in all of the conditions which were studied, except in leukemia. In the latter case there is no relationship between the nucleotide nitrogen and the number of red blood corpuscles, but there is a significant positive correlation between the nucleotide nitrogen content of the whole blood and the number of white blood cells. In other words, it would seem that the high values for nucleotide nitrogen observed in leukemia must be associated in some way with the increased white cell count. Under conditions where the number and volume of the red blood corpuscles in comparison with the white blood cells maintain the usual level, the amount of nucleotide which is present in the white blood cell is masked completely by the larger quantity of nucleotide which is confined to the red blood corpuscles.

Insofar as we are aware, there is only one analysis of the nucleotide content of the leucocytes published in the literature. Buell and Perkins[2] reported negative results for the nucleotide content of the lymphocytes taken from the thoracic duct of the dog. It therefore seemed desirable to attempt the separation of the white blood cells from the red blood corpuscles in those cases of leukemia in which small quantities of blood would give a quantity of white cells sufficient for the quantitative analysis of the nucleotide content.

The following procedure was used for the separation of the red blood corpuscles from the white blood cells. Five millimeter glass tubing was cut the size of ordinary hematocrit tubes and sealed at one end. The blood was introduced into the tubes with a syringe which had been fitted with a long needle. The tubes containing the blood were then centrifuged for 25 minutes at approximately 2500 r.p.m. After the centrifugation, the tubes contained 3 separate

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* Aided by a grant from the American Medical Association.

[1] Allen, F. W., Lucia, S. P., and Eiler, J. J., *J. Clin. Invest.*, 1936, **25**, 157.

[2] Buell, M. V., and Perkins, M. E., *J. Biol. Chem.*, 1928, **76**, 95.

and distinct layers, corresponding to red blood corpuscles, white blood cells with a few enmeshed red corpuscles, and plasma. The length of each layer in the 5 mm. tubes varies from one inch or more, dependent upon the relative volume of the red blood corpuscles and the white blood cells. The tubes were broken, with the aid of a file and gentle pressure, at the juncture between red corpuscles and white cells and also at the juncture between white cells and plasma. The cells or plasma were then aspirated from each section. If carefully done, quite an efficient separation of the three fractions may be effected. After the separation, the total volume of each cell fraction was determined, and Ringer's solution was added until the volume in each case (determined by hematocrit) was the same as that occupied by the fraction as it appeared in the original sample of whole blood.

Thus, with a sample of blood from a case of leukemia it was found possible to obtain a white blood cell suspension with a cell count comparable to that of the original blood, but with the red blood corpuscles present in very small percentages. Table I contains the results of the analyses of 2 samples of blood taken from patients with myeloid leukemia. Sample No. 1 may be taken as an example of a very marked separation of white blood cells from red blood corpuscles (less than 20,000 R.B.C./c.mm.), while sample No. 2, due to the greater diameter of the white cells with their tendency to enmesh the smaller corpuscles, is representative of one of the cases of a less marked separation (690,000 R.B.C./c.mm.). The nucleotide nitro-

TABLE I.

Partition of Nucleotide Nitrogen Between the Formed Elements of the Blood of Patients with Myeloid Leukemia.

	Nucleotide nitrogen per 100 cc., mg.	Red blood corpuscles, millions	White blood cells
Sample No. 1			
(a) Whole Blood	5.7	2.26	790,000
R.B.C. Hematocrit 16.5%			
W.B.C. " 15.5%			
(b) White Cell Fraction	3.0	0.02	500,000
(c) Red Corpuscle Fraction	1.8	1.70	8,300
(d) Plasma	1.2	—	—
Sample No. 2			
(a) Whole Blood	8.0	3.34	360,000
R.B.C. Hematocrit 20.0%			
W.B.C. " 44.0%			
(b) White Cell Fraction	6.8	0.69	464,000
(c) Red Corpuscle Fraction	3.0	2.34	13,200
(d) Plasma	0.9	—	—

gen was estimated by the quantitative method of Kerr and Blish.[3] Examination of the table shows that in cases of myeloid leukemia in which the white blood cell counts are high there is more nucleotide in the white blood cell fraction than in the red blood corpuscle fraction.

Table II contains an example of a case of myeloid leukemia upon which a serial study of the nucleotide nitrogen content has been made coincident with the regression of the white blood cell count. It will be noted that during the 20-fold decrease in white blood cell count with an approximately constant red blood corpuscle count there is a 2-fold decrease in nucleotide nitrogen content of the whole blood.

TABLE II.
Nucleotide Nitrogen Content of the Whole Blood of a Patient with Myeloid Leukemia.
(Serial samples taken during the regression of the disease.)

Date 1935	Nucleotide nitrogen per 100 cc. whole blood, mg.	Hemoglobin %	Red blood corpuscles, millions	White blood cells
10/29	9.6	50	2.93	460,000
10/31	7.3	54	3.20	390,000
11/16	4.8	67	3.68	24,000

8721 C

A Bile Fistula Procedure in the Rat.

FERN G. HARRINGTON, JOSEPH D. GREAVES* AND CARL L. A. SCHMIDT.

From the Division of Biochemistry, University of California Medical School, Berkeley.

In this laboratory the rat has been found to be a very useful experimental animal for purposes of studying bile function. Large numbers of animals may be used. Moreover, the rat is not particularly subject to infections, and special aseptic operative procedures do not need to be employed in preparing bile fistulas.

The internal bile fistula technique which has been found useful in dogs cannot be applied to the rat. This animal has no gall bladder nor a cystic duct, thus making a cholecystonephrostomy[1] impossible. The size of the structures involved prevents the making of a direct anastomosis between the bile duct and the kidney pelvis. The pancreatic ducts empty directly into the bile duct of the rat. Any bile fistula procedure must utilize only the upper portion of the bile duct so as to leave the pancreatic system intact.

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[3] Kerr, S. E., and Blish, M. E., *J. Biol. Chem.*, 1932, **98**, 193.

* E. R. Squibb and Sons Fellow.

Two bile fistula techniques in the rat have been described. In the first operation[2] an anastomosis between the bile duct and the lower colon is made with the aid of a small silver cannula. With proper care such animals live in good health for extended periods of time. The second operation[3] makes use of an artificial gall bladder which is inserted directly into the abdominal cavity. The original procedure has been modified by placing the glass bulb under the skin on the right side of the rat. The silver cannula which is inserted in the bile duct is connected to the glass vessel by means of a small parlodion tube. The second opening in the glass bulb communicates to the outside, thus permitting withdrawal of bile.

In the latter procedure rats live for only a short period of time when the bile is drained regularly. If the external opening is plugged to prevent loss of bile, the animals will survive for periods up to 6 weeks despite the extensive tissue destruction which results from the icteric condition. Administration of bile or of sodium chloride solution to the rat by stomach tube will prolong the life of the animal for a period of only 3 to 4 weeks. This, together with the danger of infection, makes the technique of no value when it is desired to prolong the experiments over periods of several months.

A more recent procedure which has been successfully employed in this laboratory consists in anastomosing the bile duct to the vas deferens by means of a silver cannula. In the rat the testes are embryologically formed well up in the body cavity and are then gradually drawn into the scrotum by shrinkage of the ligamentum gubernaculum testis. The vas deferens in the adult rat has considerable length. After sectioning the ligamentum gubernaculum testis, the vas deferens can be extended as far as the liver.

A median incision is made along the linia albia, the right testicle is drawn up from the scrotum, the ligamentum gubernaculum testis is cut, and the spermatic artery is tied. The mesentery between the vas deferens and the spermatic artery is cut so as to leave the blood supply to the vas deferens intact. The vas deferens is hemisectioned at a high level. A small silver cannula about the size of a No. 22 hypodermic needle is inserted into the duct with the aid of a stilet and tied in place with a silk suture. The vas deferens is then severed from the epididymis and the testicle is removed. The vas deferens can now be made to extend well towards the liver. The bile duct is doubly ligated at a level above the point of entrance of the major pancreatic ducts and semi-sectioned just above the ligature. The remaining end of the cannula is inserted into the bile duct. The end of the vas deferens and the bile duct are brought together and the cannula is tied in position. The bile duct is sectioned between the ligatures, and the abdomen is closed surgically.

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[1] Kapsinow, R., Engle, L. P., and Harvey, S. C., *Surg. Gynec. and Obs.*, 1924, **39**, 62.

[2] Greaves, J. D., and Schmidt, C. L. A., *J. Biol. Chem.*, 1933, **102**, 101; *Am. J. Physiol.*, 1935, **111**, 492.

[3] Sawyer, L., and Lepkovsky, S., *J. Lab. and Clin. Med.*, 1935, **20**, 958.

The success of the operation can be demonstrated by injecting a saline solution of rose bengal into the jugular vein. This dye is normally excreted through the bile and in this case should appear in the urine.

It has been possible to keep rats operated by this technique in apparent health for a period of 3 to 4 months. With cannulas of the size given above it is possible to carry out the operation on rats as small as 150 gm. body weight. Rats as small as 60 to 70 gm. may likewise be operated upon provided smaller cannulas are used. The operation eliminates the danger of infection which is present in the 2 other techniques. It permits the complete exclusion of bile from the intestinal tract. However, the technique is applicable only to male rats.

8722 P

Spectral Region of Photosensitivity in Hydroa Aestivale Seu Vacciniforme with Porphyrinuria.

HAROLD F. BLUM AND LLOYD E. HARDGRAVE.

From the Division of Physiology, University of California Medical School, Berkeley.

Many attempts have been made to produce typical lesions in Hydroa patients by exposure to light, but few, if any, have succeeded in demonstrating a definitely increased sensitivity to light. Nevertheless, it has been very generally assumed that such patients are abnormally photosensitive and that porphyrins, which are found in the urine of many of these cases, are the photosensitizing substances. The present experiments would seem to demonstrate the truth of both these assumptions, and also to explain the discrepancies in the findings of others.

A boy of 7 years, suffering from Hydroa and displaying marked porphyrinuria, was subjected to exposure of small areas of the back to bright sunlight for 30 minutes, certain of the areas being covered with glass color filters to delimit various spectral regions. At the end of this time, a distinct erythema was observed on those areas which had received any considerable amount of radiation of wave lengths between approximately 3200 Å and 4500 Å, but not on the areas from which this portion of the sunlight had been excluded by the filters. The response occurred when the radiation shorter than 3200 Å, to which normal skin is sensitive, was excluded. Thus the skin of this patient is shown to be abnormally sensitive to light, responding to the spectral region between 3200 Å and 4500 Å, to which normal skin is not sensitive.

If we examine the absorption spectrum of porphyrins, we find a nice correspondence with the region of sensitivity of our patient, which may be taken as reasonable proof that porphyrins are the photosensitizing substance. Their most characteristic absorption lies between 3200Å and 4300 Å,[1] and this absorption is much greater than that which occurs in the visible spectrum. This accounts for the fact that longer wave lengths did not produce any response in the patient, although it is quite certain that prolonged exposure would have demonstrated some sensitivity in the visible portion of the spectrum.

The patient has been receiving bi-weekly exposures to quartz-mercury arc for many months, which have never produced any abnormal reaction, and he has responded with a healthy, normal coat of tan; no immediate erythema such as we obtained with sunlight follows these regular quartz-mercury arc treatments. This is quite readily explainable since the arc produces much greater intensities of radiation shorter than 3200 Å than reach us from the sun, whereas sunlight contains much greater intensity than the arc in the region between 3200 Å and 4500 Å. Thus the quartz-mercury arc elicits only the normal delayed erythema and pigmentation, which are produced by wave lengths shorter than 3200 Å, without producing a detectable amount of the abnormal immediate erythema. Sunlight, on the other hand, elicits the abnormal response. Most of the attempts to demonstrate abnormal sensitization in Hydroa have employed the quartz-mercury arc as a source, and the results have been very conflicting, some claiming to have shown an enhanced and some a diminished sensitivity. It is probable that exposure to such a source would produce a very severe sunburn of the normal type before the abnormal hematoporphyrin response would appear, so that the latter would be masked. A few cases in which sunlight or carbon arc have been used as a source have shown results in apparent agreement with ours.

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[1] Clar, E., and Haurowitz, F., *Ber. deutsch. chem. Gessell.*, 1933, **66**, 331.

A more complete description of the experiments together with an account of artificial photosensitization with porphyrins will be given elsewhere in the near future.

8723 C

Hemolytic Phage-Bacterium Conjugates.

Jeanette D. Taranik. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

The production of hemolytic bacteriophage-bacterium conjugates was reported by Friedberger and Vallen.[1] Apparently identical aggressive conjugates were afterwards isolated from clinical material.[2] The non-hemolytic microörganisms which can be transformed into erythrolytic variants by phage-"hybridization" include *B. typhosus, B. dysenteriæ, B. paratyphosus, B. suipestifer, S. choleræ,* and *B. coli.* In order to obtain material for an immunochemic and genetic study of such "hybrids," we have tested the erythrolytic powers of 75 conjugates, formed by combining 11 local typhoid strains with 7 demonstrably active antityphoid bacteriophages. Each bacteriophage was first grown for 3 to 6 test-tube generations on each of the 11 typhoid strains. In 67 of the 77 combinations powerful lysis was noted. The 67 resulting apparently completely lysed typhoid cultures were incubated for an additional period of 48 to 72 hours. In 65 of the tubes an overgrowth with phage-resistant

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[1] Friedberger, E., and Vallen, J., *Klin. Wochenschr.*, 1923, **22**, 1649.

[2] van Loghem, J., *Centralbl. Bakt.*, 1926, **100**, 19; Sonnenschein, C., *Ibid.*, 1929, **111**, 177; 1930, **117**, 58; Kleineberger, E., *Ibid.*, 1930, **117**, 344; Bechtel, E., *Ibid.*, 1930-31, **119**; Bianchi, L., and Callerio, C., *Z. Immunitätsf.*, 1931, **72**, 155; Zimmerman, E., *Ibid.*, 1934, **82**, 495.

B. typhosus took place. Hemolytic titers of these 65 aggressive overgrowths are recorded in Table I.

TABLE I.

Hemolytic Titers of Phage-bacterium Conjugates.

65 *B. typhosus* overgrowths each inoculated into a fresh tube of 1-10 veal-broth-dilution of the corresponding bacteriophage (*i. e.*, Chamberland filtrate), incubated 24 hours, then streaked or plated on 2.5% washed-sheep-erythrocyte agar (equivalent to 5% whole blood).

Not all of the colonies on any one of the 65 resulting plates were hemolytic. All lytic colonies on any one plate, however, were of equal titer. Subcultures from lytic colonies gave pure hemolytic strains of the same titer.

Titers were estimated by comparing the hemolytic areas surrounding the colonies. Thus: ++, hemolytic zone approximately 2 mm. in diameter; +, approximately 1 mm.; ±, no lytic zone, but corpuscles laked under colony; 0, no demonstrable laking.

Bacteriophage No.	*B. typhosus* strain number											Control (No bacteria)
	20	3	21	5	6	38	8	37	22	1	4	
56	+	++*	0	0	++	±	++	+	?	0*	0	0
175	+	+	+	++	++	++	0	0	0	0	0	0
45	±	+	+	±	0*	0	0	0	0	0	0	0
8	+	0	+	+	0	0	0	0	0	0	0	0
163	++	0	0	0	0	0	0	0	+	0	0	0
53	++	0	0	0	0	0	0*	±	?	0	0	0
23	0	0	0	0	0	0	0	0	0*	0*	0*	0
Control (no phage)	0	0	0	0	0	0	0	0	0	0	0	—

* = Original typhoid culture phage-resistant. (Phage-multiplication shown by phage-titration of filtrate.)

? = Phage-resistant strain not obtained.

The table shows that only 23 (30%) of the phage-resistant *B. typhosus* variants produce demonstrable lysis, and that only 8 (10%) of them give lytic reactions equal to that of fairly active hemolytic streptococci (++). Active proliferation of bacteriophage in the 52 hemolytically negative phage-bacterium complexes was demonstrated by phage-titration of filtrates.

8724 P

Ultrafiltration of Type I Antipneumococcal Sera.

KENNETH GOODNER, FRANK L. HORSFALL, JR., AND JOHANNES H. BAUER.

From the Hospital of the Rockefeller Institute for Medical Research and the Laboratories of the International Health Division of the Rockefeller Foundation, New York.

Elford, Grabar and Fischer[1] have reported that the "antibody activity" of antipneumococcal horse-serum is associated with the "larger complex protein in the serum." Thus it was estimated by the method of optimal proportions that 75% of the antibody passed a 140mμ membrane, rather less than one percent passed an 80mμ membrane, while the filtrate from a 54mμ membrane showed no trace of antibody-activity.

The membranes used in the present experiments were prepared by the method of Elford[2] with certain modifications described by Bauer and Hughes.[3] For filtration, Type I antipneumococcal horse- and rabbit-sera were diluted 1:5 in broth. Concentrated antipneumococcal horse-serum was diluted 1:10 in the same reagent. For evaluating the amount of antibody in the filtrate the quantitative precipitation method of Heidelberger, Sia, and Kendall[4] was used. Because of the difficulties in securing large amounts of filtrates complete analyses over a wide range of amounts of polysaccharide were impossible. Therefore an arbitrary amount (0.2 mg. per cc. of original serum) of the Type I acetyl polysaccharide* was employed for each filtrate. Determinations of nitrogen in the washed precipitates were carried out by the gasometric micro-Kjeldahl method of Van Slyke.[5]

The end-points in filtration of the specific antibodies of Type I antipneumococcal horse- and rabbit-sera are shown in Fig. 1. The results are plotted in terms of the percentage of total specifically precipitable nitrogen recovered in the various filtrates against average pore-diameters. With antipneumococcal rabbit-serum no

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[1] Elford, W. J., Grabar, P., and Fischer, W., *Biochem. J.*, 1936, **30**, 92.

[2] Elford, W. J., *J. Path. and Bact.*, 1931, **34**, 505.

[3] Bauer, J. H., and Hughes, T. P., *J. Gen. Physiol.*, 1934, **18**, 143.

[4] Heidelberger, M., Sia, R. H. P., and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

* This preparation of the viscous form of the capsular polysaccharide was kindly supplied by Dr. M. Heidelberger.

[5] Van Slyke, D. D., *J. Biol. Chem.*, 1926, **71**, 235.

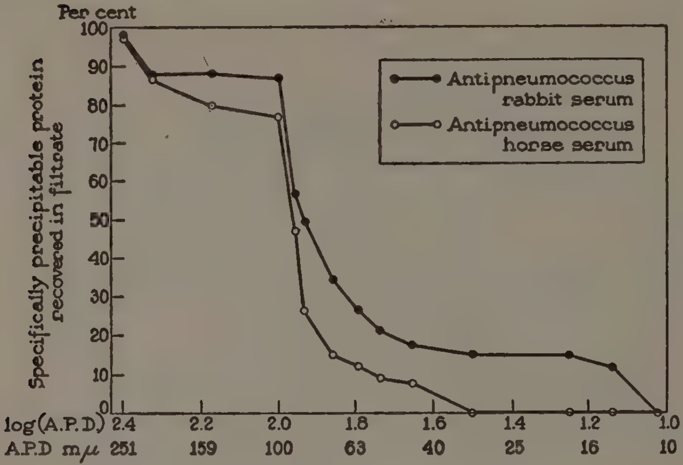

FIG. 1.

specifically precipitable protein passed through a membrane with average pore-diameter of 10.6mμ. A 13.8mμ filtrate contained 11.9% of the total specifically precipitable protein of the serum. Slightly greater amounts were then recovered as the pore-sizes were increased up to 73.0mμ. At this point the curve rose sharply until at 102.5mμ the filtrate contained 86.6% of the total amount of antibody.

With antipneumococcal horse-serum, on the other hand, the smallest pore permitting the passage of antibody was 45.2mμ. Again, between 73.0mμ and 102.5mμ, the curve rose steeply until at the latter porosity 76.7% of the antibody was recovered.

With a concentrated antipneumococcal horse-serum the end-point was relatively sharp, no antibody being recovered at 150.4mμ, while 100% was found in the filtrate at 188mμ.

These findings have been checked qualitatively on several lots of immune sera. In all experiments, both quantitative and qualitative, the rates of filtration agree well with the findings. An obvious difficulty, and one which cannot be eliminated from this technic, is that large particles tend to be fixed in the membrane and thus decrease the actual porosity of the filter.

In generalizing it may be assumed that the smallest specific antibody of antipneumococcal rabbit-serum corresponds to a pore-size of 11mμ, the smallest in horse-serum to a size of 44mμ, while both horse- and rabbit-serum have large specific aggregates corresponding roughly to a pore-size of 88mμ. Furthermore, the antibody of concentrated horse-serum requires a pore-size of approximately 176mμ. It will be noted that these figures are multiples of 11. Elford and Ferry[6] have shown that the approximate pore-size required for normal horse-pseudoglobulin in the isolated form is 11 to 12 mμ.

The sizes of these aggregates are apparently not fixed properties, for it was possible to obtain marked dispersion of equine antibodies by dilution in broth with a final NaCl concentration of 5%. The large aggregate of the concentrated serum is easily dispersed by dilution in normal horse- or rabbit-serum and even more dispersed by injection into a normal rabbit. The state of aggregation is therefore probably a function of some unknown factors of the environment. Slight variations in salinity, or variations in pH from 6.2 to 8.6, have no apparent effect.

The full import of these results, however, cannot be gained from the consideration of the amounts of specifically precipitable protein. Assuming that these particles of antibody are spheroidal and that the pore-size is a function of the diameter, it is obvious that a comparison of the volumes of the antibody-aggregates is most striking. It seems, furthermore, that the amount of nitrogen in each unit of antibody would be a function of this volume. Therefore it becomes apparent that although a very large percent of the total precipitable nitrogen is found in the larger aggregates, this may not be a true indication of the actual number of antibodies, assuming that each aggregate functions as a single antibody. On this basis of reasoning, not only is the mass of the smallest rabbit-antibody 64 times less than the smallest horse-antibody, but the ratio of small to large antibodies is very much greater in rabbit- than in horse-serum.

These observations not only offer an additional differentiation between the properties of the immune sera of these 2 animal species, but may serve to explain certain of the heretofore poorly understood properties.

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[6] Elford, W. F., and Ferry, J. D., *Biochem. J.*, 1934, **28**, 650.

8725 P

Pigment of *Bacillus violaceus.* II. Pyrrolic Nucleus of Violacein.*

Walter C. Tobie. (Introduced by J. W. Williams.)

From the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

A process for the preparation of violacein, the violet pigment of *Bacillus violaceus (Chromobacterium violaceum)* has been described.[1, 2] This pigment is of interest since several workers have attributed a respiratory function to it.[3-6] The present paper summarizes the results of a preliminary chemical examination of amorphous violacein.

Ultimate analysis. Semi-micro combustions made in duplicate on 4 to 11 mg. samples dried to constant weight in vacuum at 100°C. gave the following percentage values: C 69.08, 69.47, Av. 69.28; H 6.94, 6.63, Av. 6.79; O (by difference) 16.34, 16.59, Av. 16.45; N 4.87, 4.79, Av. 4.83; Ash 2.77, 2.52, Av. 2.65. The ash contained a considerable amount of iron. Due to the low solubility of violacein in all common solvents including melted camphor, no satisfactory value could be obtained for the molecular weight. Other workers[7, 8, 9] have reported higher nitrogen and have overlooked the presence of iron, although in one case ash was reported.[9] There is but little agreement between the values reported by different investigators for percentile composition and molecular weight. Accordingly the constitution of the pigment is still in doubt.

Solubility. Although violacein gave intensely violet solutions with many solvents, its solubility was generally very low. Its solubilities as mg. per 100 cc. of saturated solution at 23-25° were: Methyl alcohol 86, 95% ethyl alcohol 99, acetone 76, tertiary butyl alcohol 107, pyridine 389. Exposure of these solutions to strong sunlight caused the discharge of the violet color within a few hours.

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* Contribution No. 73 from the Department of Biology and Public Health, Massachusetts Institute of Technology, Cambridge, Mass.

[1] Tobie, W. C., Ph.D. Thesis, Massachusetts Institute of Technology, 1933 (unpublished), upon which the present paper is partly based.

[2] Tobie, W. C., *J. Bact.*, 1935, **29**, 223.

[3] Ewart, A. J., *J. Linn. Soc. Botan.*, 1897, **33**, 123.

[4] Shibata, K., *Jahrb. wiss. Botan.*, 1912, **51**, 179.

[5] Friedheim, E. A.-H., *Compt. rend. soc. biol.*, 1932, **110**, 353.

[6] Friedheim, E. A.-H., *Arch. sci. phys. nat.*, 1932, **14**, 125.

[7] Reilly, J., and Pyne, G., *Biochem. J.*, 1927, **21**, 1059.

[8] Kögl, F., 1932, *Handbuch der Pflanzenanalyse* (Gustav Klein Ed.), III/2 *Spezielle Analyse* II, 1410.

[9] Wrede, F., and Rothhaas, A., *Z. physiol. Chem.*, 1934, **223**, 113.

The pyrrolic nucleus of violacein. An 8 mm. Pyrex tube was drawn to a capillary 12 cm. long, bearing a small bulb with an open end. A plug of asbestos was inserted next to the capillary, followed by a layer of zinc dust and a mixture of 45 to 270 mg. of violacein with 1 gm. of zinc dust. The free end of the tube was then fire-sealed. First the zinc dust and then the zinc dust-violacein mixture was heated with a burner. Water at first collected in the capillary, but at a dull red heat yellow-brown droplets of an oil condensed in the asbestos. These were driven into the capillary and bulb by further heating. The capillary was then sealed off and the oil treated with anhydrous sodium sulphate to absorb the water. The oil darkened rapidly in contact with the air. It was soluble in ethyl ether and petroleum ether, but insoluble in 95% ethyl alcohol. Attempted boiling-point determinations by the Sewaliboff method were inconclusive, due to the rapid carbonization of the oil when heated in contact with the air. A reddish deposit appeared on the thermometer where vapors of the oil came in contact with sulphuric acid fumes from the bath, suggesting the formation of pyrrol red. For convenience in running color-tests, portions of the oil were dissolved in petroleum ether which was allowed to evaporate spontaneously in test tubes. The residue in the tubes gave strong positive tests for a pyrrol as follows: Ehrlich test (p-dimethylaminobenzaldehyde and HCl) vivid pink, vanillin and HCl intense yellow or orange, Hopkins and Cole reaction (glyoxylic acid reagent and sulphuric acid) pink. No picrate or picramate could be obtained from the oil. Similar pyrrolic reduction-products were also obtained by reduction with hydriodic acid, or with zinc dust or sodium hydrosulphite in NaOH solution. These products also gave strongly positive pyrrolic color-reactions. They showed very little volatility with steam.

Other properties of violacein. Reduction of a saturated solution of violacein in glacial acetic acid by warming with coppered granular zinc gave no pyrrol but yielded a pale straw-colored solution. On carefully neutralizing the acid and allowing the mixture to stand overnight, the original violet color reappeared. Apparently mild reduction converts violacein into a leuco base from which it is readily regenerated by the action of atmospheric oxygen. Violacein was stable in buffered alkali up to pH 11, but above that point it dissolved to a pink solution which later became brown. Strong alkali such as NaOH solution decomposed it with liberation of ammonia. HCl exerted a destructive action on violacein in alcoholic solution. In an attempt to prepare an ash-free preparation, 172 mg. of the pigment was dissolved in 300 cc. of 95% ethyl alcohol and HCl gas was passed in until the violet color had changed to green. On addition of 500 cc. of water, a dark violet precipitate separated from a brown supernatant liquid. After filtration, washing, and drying, the weight of the precipitate was only 97 mg. indicating that over 40% had been destroyed by the HCl. Although the ash was reduced, the percent of nitrogen dropped to 3.43 indicating a partial decomposition of the molecule. By passing an excess of HCl into an alcoholic solution, the violet color was completely destroyed and could not be restored by neutralization.

The production of pyrrolic products by several different methods of reduction indicates that violacein contains one or more pyrrol rings in its molecule. The fact that the pyrrolic products exhibit little volatility with steam and are soluble in ethyl and petroleum ethers but insoluble in ethyl alcohol, suggests that the pyrrolic ring or rings may possess hydrocarbon side-chains. Probably violacein is chemically similar to prodigiosin, the red pigment of *Bacillus prodigiosus* which has been shown to be a tripyrrylmethane derivative.[10] The presence of iron is of possible significance in view of the supposed respiratory function of the pigment, and may indicate an analogy to hemoglobin, chlorophyll, and other metal-pyrrol compounds.

8726 C

Influence of Certain Liver Poisons on Action of Parathyroid Extract.

DAVID M. GREENBERG.

From the Division of Biochemistry, University of California Medical School, Berkeley.

In 1932 when practically nothing was known of the mechanism of the action of the parathyroid hormone in raising the blood calcium level, this investigation was undertaken to test the hypothesis that some other tissue, particularly the liver, might be involved as an intermediary in the action of the hormone. Since then Selye,[1] and Thompson and Pugsley[2] have proposed that the hormone acts directly on bone by stimulating an osteoclastic reaction. While there is much in favor of this view, the hypothesis of an intermediating action by some other tissue has by no means been completely eliminated.

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[10] Wrede, F., and Rothhaas, A., *Z. physiol. Chem.*, 1933, **222**, 203.

[1] Selye, H., *Arch. Path.*, 1932, **14**, 60; *Endocrinology*, 1932, **16**, 547.

Thus, so far, no one has been able to demonstrate a direct action of the parathyroid hormone on bone by *in vitro* experiments. Robison and Rosenheim,[3] for example, failed to find any effect by parathyroid extracts on the *in vitro* calcification of bone.

Furthermore, certain substances which produce liver damage, markedly reduce the rise in serum calcium normally produced by injections of parathyroid extract. In the early stages of this work[4] it was observed that acute intoxication with elementary phosphorus practically obliterated the serum calcium raising effect of the parathyroid hormone. This observation was also made independently and reported at about the same time by Nitzescu.[5]

The effects of the liver poisons carbon tetrachloride and hydrazine as well as of phosphorus have now been determined. The results obtained show that while phosphorus intoxication almost obliterates the increase in serum calcium normally produced by the injection of parathyroid extract, hydrazine intoxication causes a less marked although definite retardation and carbon tetrachloride causes no appreciable decrease.

It has been suggested by Thomson and Collip[6] that the action of phosphorus might well be the result of a toxic injury to the bone cells. The experiments with benzol were undertaken to subject this idea to an experimental test, since benzol is a well known bone marrow poison.

Dogs weighing between 12 and 15 kilos were used in the work. With each animal a control experiment was first performed by subcutaneously injecting 500 Hansen units of parathyroid extract, Lilly,* and then following the serum calcium curve for the next 24 hours. Acute intoxication by phosphorus, carbon tetrachloride and hydrazine was induced in the manner described below. Phosphorus intoxication was produced by injecting daily 5 to 10 mg. of yellow phosphorus in oil (10 mg. P per ml. of olive oil) subcutaneously for a period of from 2 to 4 days prior to the injection of 500 Hansen units of Parathyroid Extract, Lilly, and then following the change in the serum calcium curve.

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[2] Thomson, D. L., and Pugsley, L. I., *Am. J. Physiol.*, 1932, **102**, 350.

[3] Robison, R., and Rosenheim, A. H., *Biochem. J.*, 1934, **28**, 684.

[4] Greenberg, D. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 721.

[5] Nitzescu, I. I., *Compt. rend. soc. biol.*, 1932, **110**, 1141.

[6] Thomson, D. L., and Collip, J. B., *Ann. Rev. Biochem.*, 1933, **2**, 231.

* The Parathyroid Extract used in these experiments was generously furnished by Eli Lilly and Co.

As recommended by Minot and Cutler,[7] the carbon tetrachloride was administered by stomach tube, daily doses of 40 ml. each being given for periods of 2 days. Hydrazine intoxication was produced by the subcutaneous daily injection of 10 ml. portions of 2% aqueous hydrazine sulfate for periods of 6 days prior to the administration of parathyroid extract. Each of the liver poisons was usually administered for several intoxication periods spaced about two weeks apart. In the course of these intoxications, the animals developed jaundice, bile appearing in the blood and urine. Autopsy revealed manifestations of liver damage in each instance.

Benzol intoxication was induced after the manner described by McGavack[8] by injecting 4 ml. of a mixture of equal parts of benzol and olive oil twice daily for 4 days prior to the administration of parathyroid extract. It was not feasible to repeat these experiments on the same animal as was done with the other toxic materials, because huge open sores developed on the skin at the sites of injection. The benzol intoxicated animals became dropsical, fluid being found at autopsy in the chest cavity. No significant drop in the red corpuscle count was observed during the duration of these experiments.

Representative experimental results obtained with each of the toxic substances subjected to test, are plotted in Fig. 1. In the block figures, the initial serum calcium level at the start of an experimental period is indicated by the white portion and the maximum serum calcium level produced following injection of parathyroid extract, by the cross hatched portion. The control periods are distinguished from the intoxication periods by the reversed direction of the cross hatching. The lettering along the abscissa refers to the designation assigned to the experimental dogs.

From Fig. 1 it is apparent that phosphorus profoundly checks the rise in serum calcium normally produced by the parathyroid hormone. With dog Bl, in the third intoxication period, injection of parathyroid extract actually lowered the serum calcium level. Hydrazine also exerts a considerable and consistent effect in lowering the magnitude of the increase in the serum calcium level. On

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[7] Minot, A. S., and Cutler, J. T., *J. Clin. Invest.*, 1928, **6**, 369.

[8] McGavack, T. H., *J. Am. Institute of Homeopathy*, 1928, **21**, 461.

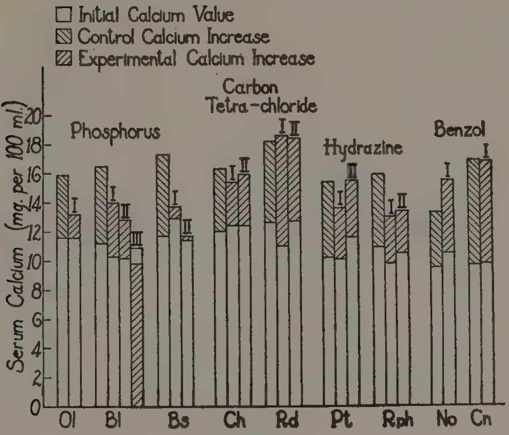

FIG. 1.

The Effect of Certain Liver Poisons on Parathyroid Action.

Each group of block figures represents the results of an experimental series on a dog. The designation of each dog is given by the lettering under the blocks along the abscissa. The initial serum calcium level at the start of each experiment is represented by the height of the white portion of the block; the maximum rise in serum calcium produced by the injection of 500 Hansen units of parathyroid extract by the cross hatched portion. The control and intoxication periods, as shown above, are cross hatched in reverse directions.

The dogs employed in these experiments weighed between 12 and 15 kilos. Intoxication with each of the substances employed was induced in the following manner:

Phosphorus: Dog Ol, Bl, and Bs were subcutaneously injected with 5 to 10 mg. of yellow phosphorus in oil for two to four-day periods.

Carbon tetrachloride: 40 ml. doses per day of carbon tetrachloride were administered by stomach tube to dogs Ch and Rd for two-day periods.

Hydrazine: 10 ml. portions of 2 per cent aqueous hydrazine sulfate were subcutaneously injected into dogs Pt and Rph daily for six-day periods.

Benzol: Dogs No and Cn received twice daily injections of 4 ml. portions of a mixture of equal parts of benzol and olive oil for 4 days.

the other hand, it appears from Fig. 1, that carbon tetrachloride exerts none or only a slight reducing effect on the calcium rise.

No definite explanation is available for either the inhibiting effect of the substances inducing liver damage on the parathyroid action, nor for the differentiation in the action of the 3 test substances. Although profoundly toxic effects were produced by each of the 3, the possibility exists that different degrees of liver damage were produced by phosphorus, hydrazine and carbon tetrachloride. Also possibly different liver cells of the liver are injured by the action of phosphorus than by carbon tetrachloride. This suggestion is supported by other experimenters.[9, 10] Haloff[9] states that chloroform and carbon tetrachloride produce a fatty degeneration of the liver, while phosphorus attacks the Kupfer cells more.

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[9] Haloff, G. A., *Arch. Exp. Path. Pharm.*, 1928, **134**, 168.

[10] Marshall, E. K., and Rowntree, L. G., *J. Exp. Med.*, 1915, **22**, 333.

The observation that benzol intoxication does not interfere with the rise in serum calcium induced by parathyroid extract, indicates that the mechanism of action of phosphorus and hydrazine is not through an injurious action on the bone cells.

Summary. 1. In acute intoxication experiments with the liver poisons, phosphorus, hydrazine and carbon tetrachloride it was found that phosphorus intoxication almost eliminates the serum calcium rise normally produced by injection of parathyroid extract, hydrazine causes a less marked rise and carbon tetrachloride has no appreciable retarding effect. 2. Since the bone-marrow poison, benzol, causes no alteration in the blood calcium curve following injection of parathyroid extract, the action of phosphorus and hydrazine appears not to be through an injurious action on the bone cells.

8727 C

Effect of Ultracentrifuging on Oxygen Consumption of the Eggs of *Ascaris suum*, Goeze.

G. C. HUFF AND E. J. BOELL. (Introduced by J. H. Bodine.)

From the Zoological Laboratories of Drake University and the State University of Iowa.

That the maintenance of structural integrity is of prime importance for the normal functioning of a large part of the oxidative processes of the cell has been demonstrated by a number of workers.[1-5] Bodine and Boell[6, 7] have shown that the normal activity of much of the so-called cyanide sensitive respiratory mechanism (indophenol oxidase system) is interfered with as the result of the structural disorganization of the cell. Recently Beams and King[8]

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*Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

[1] Warburg, O., and Meyerhof, O., *Pflüg. Arch.*, 1912, **148**, 295.

[2] Warburg, O., *Pflüg. Arch.*, 1914, **158**, 189.

[3] Runnström, J., *Protoplasma*, 1930, **10**, 106.

[4] Quastel, J. H., and Wheatley, A. H. M., *Bioch. J.*, 1932, **26**, 726.

[5] Quastel, J. H., *Bioch. J.*, 1932, **26**, 1685.

[6] Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1936, **7**, 455.

[7] Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1936 (in press).

[8] Beams, H. W., and King, R. L., 1936 (in press).

found that cleavage in the eggs of *Ascaris suum* was not affected even though the eggs were subjected to forces of 150,000 to 400,000 times gravity (obtained through the use of the Beams[9] ultracentrifuge). Although such eggs were completely stratified into a centrifugal cap of heavy yolk material, a middle band of clear protoplasm, and a light layer of lipoidal material, cleavage rate in centrifuged eggs was not noticeably different from that in normal eggs.

The purpose of the experiments reported herein was to determine the effect of ultracentrifuging on the oxygen consumption of the eggs of *Ascaris suum*.

The eggs used were obtained fresh from adult worms, expressed into saline, washed repeatedly to remove surplus albumin, and stored at 5°C. until needed. In the centrifuge experiments a concentrated suspension of eggs was rotated for 30 minutes at a force of 150,000 times gravity. Oxygen consumption was measured by means of sensitive microdifferential manometers ($K_{O_2} = 0.160$). In an experiment one drop of a concentrated suspension of ova (containing ca. 5,000 eggs) was supported by a convenient hanger in the manometer vessel. Ten percent KOH was used to absorb CO_2. In the experiments involving the use of cyanide a freshly prepared KCN solution was neutralized with HCl to pH 7.0 and diluted to 0.001 molar immediately before use. Eggs were washed 3 times with cyanide and were then supported in the manometer in cyanide. KOH-KCN mixtures for absorbing CO_2, as recommended by Krebs[10] and van Heyningen,[11] were used. For each sample of eggs tested, the respiratory rate of normal eggs was determined in duplicate; the effect of centrifuging or cyanide, in duplicate or triplicate. O_2 uptake was measured at 30° ± 0.02°C. over a 4 to 6-hour interval. At the end of the experimental period the eggs were flooded over a squared slide and counted. The respiratory rate (Q_{O_2}) was calculated in terms of mm.^{3} O_2 per hour per 10^6 eggs.

The results of a typical experiment are presented in detail in Table I; a summary of all experiments is contained in Table II.

Table I shows that the Q_{O_2} for centrifuged eggs was much greater during the third and fourth hours than during the preceding 2, an indication that at least partial recovery from the effects of centrifuging had taken place. Twenty-four hours later such eggs showed complete reorganization of cellular components and many were in the 2-cell stage.

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[9] Beams, J. W. and Pickels, E. J., *Rev. Sci. Inst.*, 1935, **6**, 299.

[10] Krebs, H. A., *Bioch. J.*, 1935, **29**, 1620.

[11] Heyningen, W. E. van, *Bioch. J.*, 1935, **29**, 2036.

TABLE I.

Time, Hours	Q_{O_2} (mm³ per hour per 10^6 eggs) Control	10⁻³ M KCN	Centrifuged	Centrifuged + 10⁻³ M KCN
1	97.6	35.7	25.5	28.0
2	97.6	25.9	22.3	28.0
3	102.9	13.0	37.3	16.0
4	113.4	13.0	67.0	19.2

TABLE II.

Experimental condition	Ave. Q_{O_2}	% respiration. Control = 100	Remarks
Control	102.3	100	Cleavage after 24 hrs.
10^{-3} M KCN	11.3	11	Cleavage 48 hrs. after removal of cyanide.
Centrifuged	22.3	21.8	Cleavage after 24 hrs.
Centrif. + 10^{-3} M KCN	12.8	12.5	Cleavage 48 hrs. after removal of cyanide.

Although cyanide markedly depressed the respiration of normal eggs it had no effect on the O_2 uptake of centrifuged eggs. Thus centrifuging seems to exert its effect primarily on the cyanide sensitive respiratory mechanisms.

The action of cyanide is rapid, maximal inhibition being apparent within 2 hours after application. With unneutralized cyanide Zawadowski[12] has claimed that cyanide produces maximal inhibition only after 5 to 9 hours in the eggs of *A. megalocephala*. It is noteworthy that the increase in respiration with time does not occur in centrifuged eggs in the presence of cyanide although physical reorganization apparently is not interfered with.

The results indicate that respiration in Ascaris eggs is depressed as a result of the disorganizing effect of ultracentrifuging and that apparently the respiratory processes and those relating to cleavage are not intimately associated.

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[12] Zawadowski, M. N., *Biol. Gen.*, 1926, **2**, 442.

8728 P

Effect of Methylene Blue on Respiration of Blocked and Developing Embryonic Cells.*

J. H. Bodine and E. J. Boell.

From the Zoological Laboratory of the State University of Iowa.

We[1] have recently shown that actively developing embryos of the grasshopper, *Melanoplus differentialis,* have a respiratory rate 2 to 3 times higher than do embryos whose activity is to a large extent blocked as the result of diapause. The average respiratory rate for embryos immediately before and after diapause ranges between 0.1 and 0.15 mm.^{3} per embryo per hour; the respiratory rate of blocked embryos averages approximately 0.05 mm.^{3} per embryo per hour. In these 3 states of development the embryos are morphologically identical—moreover, the dry weight of the embryos is the same in each case, *viz.,* ca. 0.07 mg.

In an attempt to gain some information regarding the nature of the block to respiration which occurs during diapause, the effect of methylene blue on the respiratory activity of blocked and developing embryos has been studied.

The embryos were freed from egg membranes and extra-embryonic materials and were suspended in a medium consisting of 0.9% NaCl, 0.02% each of KCl and $CaCl_2$ and 0.5% dextrose. The solution was buffered with phosphate (final concentration 0.01 molar) to pH 6.3—the pH of the perivitelline fluid of the egg.

Oxygen consumption of the embryos was determined by means of sensitive microdifferential manometers at $25° \pm 0.02°C$. Methylene blue solutions were prepared fresh daily in the saline-dextrose solution described above. In the use of cyanide the technique outlined by Huff and Boell[2] was followed.

The data in Table I show that methylene blue has only a slightly stimulating effect on the oxygen consumption of developing embryos, the average increase in respiration approximating 25%. On the other hand, the addition of methylene blue to blocked embryos practically doubles the rate of oxygen uptake. Nearly maximal stimulation is obtained with 3×10^{-5} molar methylene blue. When the concentration of the dye is increased beyond 15×10^{-5} molar,

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* Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

[1] Bodine, J. H., and Boell, E. J., 1936 (in press).

[2] Huff, G. H., and Boell, E. J., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 626.

TABLE I.

Type of Embryo	O_2 uptake per hour Control mm.^{3}	 M.B. added mm.^{3}	% increase in M.B.	Molar conc. of M.B.
Pre-diapause	.100	.102	2	3×10^{-5}
Diapause	.050	.096	93	3×10^{-5}
”	.057	.117	105	15×10^{-5}
Post-diapause	.106	.131	24	3×10^{-5}
” ”	.102	.132	29	6×10^{-5}

TABLE II.

Experimental Condition	mm^3 O_2 uptake per hour Control	 M.B. added	 KCN added	 KCN + M.B. added
10^{-4} M KCN 6×10^{-5} M M. Blue	.102 (100%)	.132 (129%)	.046 (45%)	.132 (129%)

respiration is usually stimulated to a lesser extent. It is noteworthy that methylene blue raises the respiration of blocked embryos to the level which is characteristic for normally developing embryos. (Runnström.[3])

When the respiration of developing embryos is inhibited by cyanide the addition of methylene blue restores respiration to the normal level. As is indicated in Table II 6×10^{-5} molar methylene blue can completely annul the inhibiting effect of 10^{-4} molar cyanide.†

The above facts suggest that methylene blue can substitute for that portion of the respiratory mechanism which is inactivated either naturally as the result of the diapause block or artificially through the action of cyanide.

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[3] Runnström, J., *Protoplasma*, 1930, **10**, 106.

† No antagonism between carbon monoxide and methylene blue has been observed.

8729 C

Sonic Extraction of Labile Bacterial Constituents.

LESLIE A. CHAMBERS AND EARL W. FLOSDORF. (Introduced by D. W. Bronk.)

From the Johnson Foundation for Medical Physics and the Departments of Bacteriology and Pediatrics, University of Pennsylvania.

Recent investigations have indicated that the killing of certain bacteria by available agents destroys or alters at least some of their antigenic constituents. Thus the investigations of Felix[1] have revealed a labile virulence (Vi) antigen detected only in living, virulent strains of *Eb. typhi.* Similarly, Mudd, Pettit, Lackman and Czarnetzky[2] have found that the antibody responsible for the phagocytosis of a strain of *Streptococcus hemolyticus* is elicited in rabbits by the injection of living organisms and that the living organisms will absorb the antibody from immune serum so produced. The corresponding bacterial antigen itself has resisted all ordinary methods of separation or fractionation.

Since it has been shown that sonically killed bacteria are disintegrated[3, 4, 5] and since some heat labile proteins are not denatured by the sonic treatment,[6] it appeared possible that labile but important antigens such as those referred to above might be extracted by an adaptation of the method of sonic disintegration. The purpose of this paper is to describe the apparatus and technique which we have used; to make brief mention of the antigens obtained thus far; and to point out some implications of these observations.

We have used a modified Pierce magnetostriction oscillator as the source of intense vibrations of about 8900 c.p.s. A similar oscillator has been described by Gaines[7] and was used by Chambers and Gaines[3] in their earlier work. The circuit design in our apparatus differs somewhat from that of Gaines but the essential features of the sound source are the same. The electrical circuit we have used is shown in Fig. 1.

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[1] Felix, A., and Pitt, R. M., *Lancet*, 1934, **2**, 186; Felix, A., and Bhatnagar, S. S., *Brit. J. Exp. Path.*, **16**, 422.

[2] Mudd, S., Pettit, H., Lackman, D., and Czarnetzky, E. J., Proc. Am. Assn. Path. and Bact., Boston Meeting, *Am. J. Path.*, 1936 (in press).

[3] Chambers, L. A., and Gaines, N., *J. Cell. and Comp. Physiol.*, 1932, **1**, 451.

[4] Harvey, E. N., and Loomis, A. L., *J. Bact.*, 1929, **17**, 375.

[5] Williams, O. B., and Gaines, N., *J. Infect. Dis.*, 1930, **47**, 485.

[6] Chambers, L. A., and Flosdorf, E. W., *J. Biol. Chem.*, 1936, **114**, 75.

[7] Gaines, Newton, Physics, 1932, **3**, 209.

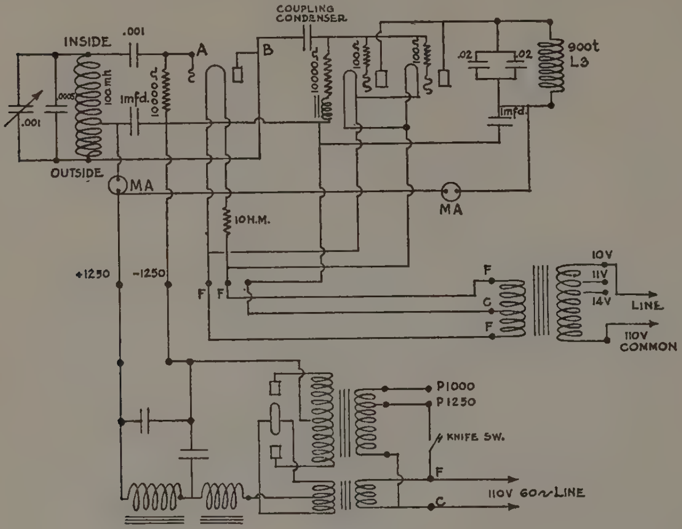

FIG. 1.

Circuit diagram of the electrical oscillator. The polarizing circuit is not included.

The arrangement of the vibrating element with relation to polarizing and driving coils is illustrated in Fig. 2. The vibrator itself is a tube of No. 20 Stubbs gauge, seamless, cold-drawn nickel tubing 24.6 cm. in length and 1.875 cm. in outside diameter. The upper end of the tube is closed by a welded nickel cap as in the original Gaines device, but we have found it unnecessary to slit the lower half of the tube. Adequate radiation of heat due to eddy currents is possible, as described below, when no such kerf is present.

The nickel tube is actuated by 1250 volt A.C. carried in the coil about the midpoint of the tube. Such a position of the driving solenoid makes possible a symmetrical distribution of the polarizing field provided by the independently excited C shaped electromagnetic circuit. The polarizing coil in our apparatus was designed to provide 5000 ampere turns, much in excess of the actual requirement. Strength of polarizing field is not critical.

Operation of the vibrating unit consists in simple tuning of the electrical oscillator to resonance with the nickel tube. In the present experiments the vibrator was of such size and shape that the system was in resonance at 8900 c.p.s. At that frequency the vibration amplitude was of the order of 0.001 cm. or just below the critical amplitude required for rupture of the nickel.

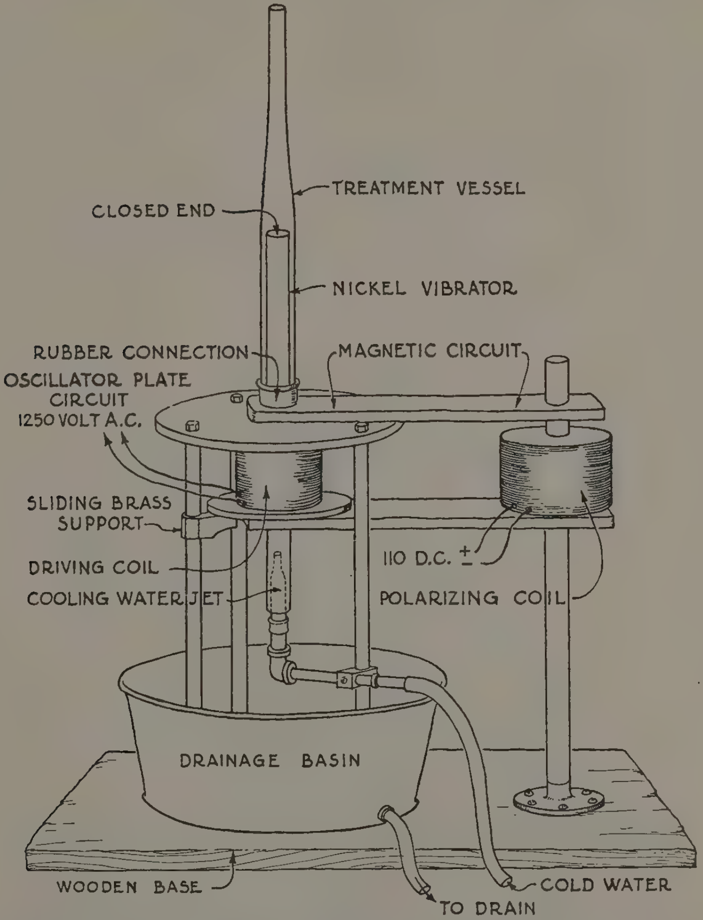

FIG. 2.

Diagram showing arrangement of driving and polarizing coils relative to the nickel vibrator. The water cooling arrangement is also represented.

Exposure of bacterial suspensions was accomplished by immersion of the capped upper end of the nickel vibrator in the suspension contained in a cylindrical glass vessel as shown in Fig. 2. The vessel was of about 2.5 cm. internal diameter and extended from the middle of the vibrator, where it was connected by a flexible rubber joint, to a point about 20 cm. above the capped end. The upper portion of the vessel was in the form of an inverted cone with an apical aperture small enough to permit convenient sterile closure, but large enough to allow passage of a pipette. The entire assembly, nickel tube, glass vessel, and rubber joint, was easily removable from the coil support and could therefore be sterilized by autoclaving, and it could be filled and emptied under aseptic conditions.

Of primary importance in the present investigation was the problem of maintaining a low temperature within the bacterial suspension during the entire period of exposure. This was accomplished by means of a jet of cold water projected into the open lower end of the vibrating tube in a manner similar to that previously described. The temperature of the water was about 10° and an adequate flow for maintenance of a 15° level in the suspension was found to be about 2 liters per minute.

Labile constituents with antibody combining power have been identified in the supernatant liquid obtained by centrifuging sonically disintegrated *Eberthella typhi* and *Streptococcus hemolyticus*.

The *Eb. typhi* culture was a mixture of 3 virulent strains recently isolated from carriers and from a severe case. Each strain contained the "Vi" antigen. The organisms were cultured on infusion agar (pH 7.2), and the washings from 8 Roux bottles suspended in physiological saline and diluted to a total volume of 20 ml. were used for each sonic extraction. Vibration of such a heavy suspension resulted in a rapid decrease in opacity until at the end of 45 minutes the liquid was quite translucent but not completely clear. Tests subsequent to exposure showed relatively very few viable cells. Microscopically it was possible to distinguish a few intact organisms and a few recognizable bacterial fragments but the bulk of the suspended material was unorganized.

After vibration for 45 minutes the suspension was centrifuged to remove the debris and bacterial residue. With the collaboration of Mr. R. E. Greaves it has been found that the resulting clear liquid will precipitate the "Vi" antibody in sera from which all somatic and flagellar antibodies had previously been absorbed. After precipitation with the sonic material this absorbed serum will no longer cause the agglutination of organisms containing the "Vi" antigen. A full report of the identification of the extracted antigen will be published elsewhere.

Of still greater interest is the new antibody-combining constituent extracted in similar manner from *Streptococcus hemolyticus*. A virulent human strain (Griffith's Type I) in the mucoid phase and of recent animal passage was used. Organisms in 7.5 liters of 18-hour infusion broth culture were separated by centrifugalization yielding about 2 ml. of solid organisms. They were then suspended in 18 ml. of 0.85% NaCl. The final concentration of living cells was of the order of 10^{11} per milliliter.

Such a suspension was exposed to vibration in the glass treatment vessel for one hour. It was then centrifugalized to remove the debris. By this operation about 18 ml. of clear liquid was recovered. During vibration the same clearing of the suspension noted in the case of *Eb. typhi* occurred, but to a lesser degree. Microscopic observation showed a rapid shattering and dispersion of clumps and chains, followed by a less rapid disintegration of the individual cells. The disintegrative process has been studied in detail by one of us (Chambers) and full description is in preparation.

As was true in the case of the typhoid bacillus, the streptococcus suspension was not rendered perfectly sterile by one hour of vibration. The survival curve is typically logarithmic, its slope being a function of the sound intensity—in other words, of the amplitude when the frequency is constant. Because of the lack of sterility it was necessary to pass the clear supernatant liquid rapidly through a Berkefeld filter under conditions permitting a minimum adsorption of active material. This was accomplished by dilution of the recovered 18 ml. with saline to a volume of 72 ml. before filtration.

The soluble protein contained in the filtrate will absorb from immune serum the antibody responsible for phagocytosis as shown by Mudd, Pettit, Lackman and Czarnetzky, who will publish elsewhere a full account of their work. The substance is destroyed by formalin and is inactivated at pH 10. It is also inactivated by heating for 30 minutes at 56°; similarly exposure of a suspension of living streptococci of this strain to 56° for 30 minutes suffices for sterilization. Furthermore the lability of the antigen is such that it deteriorates completely during three days of storage at 2° to 4°C. It may, however, be stored in "lyophile"[8] form after rapid drying from the frozen state and it will be very unusual if we do not find that it can be stored at least a year in this condition without deterioration.

The extraction of labile substances from living bacterial cells by sonic comminution appears to be a tool of considerable usefulness in immunology. The substances extracted thus far may prove of great practical importance if studies now under way show that the extracts will elicit antibody formation in the experimental animal. The implications are obvious. Production of antibodies against antigens that are destroyed by previously employed means of vaccine production is of more than theoretical interest since it is these antibodies which may be of the greatest importance in combatting infection by the more virulent strains of organisms.[1, 9] On the other hand, should no results of immediate practical importance result from the extraction of the 2 labile substances, the value of the sonic method as a research tool in immunology and bacteriology has already been demonstrated. For example, known methods of mechanical disintegration, as in the ball mill, are too slow in their action to enable the extraction of constituents which deteriorate completely during three days of storage at low temperature. Only by rapid sonic disintegration combined with lyophile preservation has it been possible to obtain the new *S. hemolyticus* material in sufficiently stable form for use in experiments which require more than a day for completion.

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[8] **Flosdorf, E. W., and Mudd, S., *J. Immunol.*, 1935, 29, 389.**

8730 C

Myohemoglobin Content of the Hypertrophied Heart of the Anemic Rat.

D. W. COWAN AND L. C. BAUGUESS. (Introduced by H. M. Hines.)
From the Departments of Physiology and Biochemistry, State University of Iowa.

The anemia which develops in rats raised on an iron and copper-free diet is accompanied by marked cardiac hypertrophy and significant chemical changes in the myocardium. A study of the muscle hemoglobin in the hearts of these animals is presented in this paper.

Most of the methods used were described in an earlier report.[1] In the present series, the animals were quickly opened along the mid-line while under light ether anesthesia, a cannula inserted into the inferior vena cava, and the point forced up past the level of the diaphragm. The heart was thus perfused with normal saline containing 5% glucose at 37°C. until it ceased to beat. At this time, the fluid returning from the heart was practically free of blood hemoglobin as indicated by lack of color. The ventricles were then carefully severed from the body at the atrioventricular ring. They were opened immediately, blotted dry, weighed and analyzed for muscle hemoglobin by the procedure described by Whipple.[2] Blood

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[9] Day, H. B., *J. Path. and Bact.*, 1933, **37**, 169.

[1] Cowan, D. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 417.

[2] Whipple, G. H., *Am. J. Physiol.*, 1926, **76**, 693.

hemoglobin determinations were made by the method of Sahli. There were 14 anemic rats and 15 littermate controls. The results are shown in Table I.

TABLE I.
Data Comparison of Anemic Rats with Litter Mate Controls. Each Value Is Followed by Its Standard Deviation.

	Litter mate controls	Anemic rats
Blood hemoglobin, %	16.5 ± 1.1	4.1 ± 0.8
Ventricular wt., mg.	429.3 ± 76.3	820.1 ± 136.4
Body wt., gm.	160. ± 28.	92. ± 19.
Ventricular wt./body wt. ratio	2.7 ± 0.38	9.07 ± 1.45
Muscle hemoglobin in ventricles, mg. %	389. ± 60.	363. ± 58.
Total muscle hemoglobin in ventricles, mg.	1.63 ± 0.14	2.90 ± 0.25
Total muscle hemoglobin in ventricles, mg./100 gm. body wt.	1.04 ± 0.18	3.27 ± 0.67

The concentration of muscle hemoglobin in the myocardium is not significantly different in the 2 series. The total amount of muscle hemoglobin is nearly twice as great for the anemic rats, owing to the great hypertrophy present in these cases. It is evident that in spite of the severe anemia, growing rats have the ability to store manufactured muscle hemoglobin in their hearts in amounts not only sufficient to meet the demands of normal heart growth, but indeed to maintain normal muscle hemoglobin concentration during the development of abnormal hypertrophy.

8731 C

Staphylococcal Antihemolysin-Titers Following Staphylococcal Toxoid in Chronic Osteomyelitis.

John E. Blair and Frances A. Hallman.

From the Laboratory Division, Hospital for Joint Diseases, New York City.

The progress of immunization following the therapeutic administration of staphylococcal toxoid to patients with staphylococcal infections may be followed in the laboratory by the titration of staphylococcal antitoxin in the sera of these individuals. It is the purpose of this communication to record the serologic response, as measured by repeated titrations of antihemolysin (antitoxin?), in a series of 38 patients with chronic osteomyelitis who received therapeutic injections of staphylococcal toxoid.

For the purpose of comparison, it is essential to know the amount of circulating antitoxin in the sera of normal individuals, and of persons with chronic staphylococcal osteomyelitis before treatment with toxoid. A common basis for reference has been made available by the establishment of a provisional International Standard Staphylococcal Antitoxin.[1] The range of staphylococcal antihemolysin in normal, healthy individuals has been reported as about ⅓ to 1 standard International Unit (I.U.) by Dolman,[2] as 0.4-0.7 to 2 I.U., by Murray,[3] as 0.15-1.5 by Nélis and Poncelet,[4] and as 1 or less by Blair and Hallman.[5]

The general opinion is recorded in the literature that sera from cases of osteomyelitis possess antihemolytic titers definitely above the normal range. Thus, in 59 cases of subacute and chronic osteomyelitis, Dolman[2] reported that titers of 5-6, and occasionally of 10, I.U. were commonly obtained. Murray[3] found an average initial titer of 11.7 I.U. in 7 cases of osteomyelitis. In 5 cases reported by Nélis and Poncelet,[4] titers of 0.8-1.5 I.U. were found in 3 and titers of 3 or more units in 2. Blair and Hallman[5] have demonstrated, in a series of 80 patients with chronic osteomyelitis, that the titers of sera of these persons generally fell within the range established as normal, that is, 1 I.U. or less. Only one-fifth of the sera from these individuals had an initial titer definitely above normal, namely 3 or more units.

In the present work, staphylococcal toxoid was administered to a group of 38 patients with chronic staphylococcal osteomyelitis. The majority of the patients presented frank bone-lesions which were subjected to radical saucerization, followed by maggot-therapy, during the administration of the toxoid. The staphylococcal toxoid employed was supplied through the courtesy of Drs. A. F. Coca and E. F. Roberts, of the Lederle Laboratories. Following an initial injection of 20 "units" of toxoid, the dosage was gradually increased in 10 successive subcutaneous injections to a maximum of 1000 "units". The maximal dose was repeated in most of the cases for 10 or 15 injections. A "unit" of staphylococcal toxoid is described by the manufacturer as "the toxoid obtained from a dermonecrotizing unit of toxin (the least amount of toxin which on intradermal injection in a susceptible rabbit will produce an erythema with a central necrosis at least 5x5 mm. in diameter)".* An average of 10515 "units" of toxoid was administered, in an average of 17.9 injections, over periods varying from about 1 to 4 months. The administration of toxoid, and the clinical management of the cases was done under the supervision of Dr. Joseph Buchman, of the Department of Orthopedic Surgery, service of Dr. Samuel Kleinberg.

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[1] Hartley, P., and Llewellyn Smith, M., *Quart. Bull. Health Organis.*, League of Nations, 1935, Jan., 68.

[2] Dolman, C. E., *Lancet*, 1935, **1**, 306.

[3] Murray, D. S., *Lancet*, 1935, **1**, 303.

[4] Nélis, P., and Poncelet, F., *Compt. rend. Soc. biol.*, 1935, **118**, 312.

[5] Blair, J. E., and Hallman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 382.

The course of immunization of each patient was followed in the laboratory by the determination at varying intervals of the antihemolytic titer of the patient's serum. An initial titration was done in each case prior to the beginning of toxoid-therapy, and from 2 to 9 titrations were done during and after the course of treatment. The titrations were originally done by determining that dilution of serum which just inhibited hemolysis of 1% rabbit erythrocytes by one M.H.D. of staphylococcal toxin, after incubation for one hour at 37°C. Subsequently, a sample of the International Standard Antitoxin was obtained through the courtesy of Dr. Hartley, and after suitable preliminary tests, our sera were titrated in terms of that standard. The titers recorded in this paper are in International Units.

Preliminary to titrating sera in terms of the International Standard, the toxin employed was first standardized. This was done by setting up a series of tubes, each containing decreasing quantities of toxin, made up to 0.5 cc. with physiologic salt-solution, and one unit of Standard Antitoxin (so diluted that one unit was contained in 1 cc.). After incubation at 37°C. for 15 minutes, 0.5 cc. of a twice-washed suspension of 4% rabbit-erythrocytes was added to each tube, giving a final concentration of 1%, and incubation was continued for one hour at 37°C. The amount of toxin which just caused hemolysis represented the "test-dose" of toxin.

In titrating serum, one cc. each of varying dilutions of the serum was placed in a series of tubes, and to each was added the test-dose of previously standardized toxin, made up to 0.5 cc. After incubation for 15 minutes at 37°C., 0.5 cc. of 4% erythrocytes was added to each tube, the tubes were shaken, and incubated for one hour at 37°C. The dilution at which hemolysis was just discernible represented the titer of the serum. As controls, from 4 to 6 previously titrated sera were always run with each lot of new sera. Agreement of the controls with previous results was required before accepting the titrations of the new sera.

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* This description is given in a folder accompanying each vial of toxoid.

In both tests we have found it convenient to centrifugate the tubes when they were removed from the waterbath for final reading, as the clear supernatants allowed more precise reading of small degrees of hemolysis. It is essential to use rabbit-erythrocytes in all hemolytic titrations of staphylococcal toxin and antitoxin.

The toxin used was prepared from the "Ha" strain of *Staphylococcus aureus,* isolated by Dr. Burky. Our culture was obtained through the courtesy of Dr. Roberts, of the Lederle Laboratories. Cultures were grown in semisolid (0.3%) agar, in an atmosphere of 30% CO_2 for 48 hours at 37°C. The culture was then drained through gauze, and filtered through a Seitz filter. The potency of the filtrate (toxin) was demonstrated by dermonecrotic and lethal tests on rabbits, and by hemolytic tests.

We found a definite rise in antihemolytic titer in the sera of all of the 38 cases following administration of staphylococcal toxoid. The increase from initial to maximal titers is recorded in Table I.

TABLE I
Increase of Antihemolytic Titer Following Staphylococcal Toxoid-Therapy in Chronic Osteomyelitis.

No.	Initial	Maximal Titers														
Sera	Titer	1.1	2.2	3.3	4.4	5.5	6.6	7.7	8.8	10	12.2	13.3	14.4	15.5	17.7	28.8
8	0.25		2		2	1	3									
9	0.5	2		2	1	1	1		1						1	
9	1.1				3	2			1	2		1				
5	2.2				1			1		2			1			
3	3.3						2							1		
2	4.4													1		1
1	5.5										1					
1	6.6															1
38		2	2	2	7	4	6	1	2	4	1	1	1	2	1	2

Titers are recorded in terms of International Units.

The results obtained confirm the findings of Dolman in cases of osteomyelitis, and of Murray and others in superficial staphylococcal infections. These workers have found that the titer usually increases 5- to 10-fold subsequent to the administration of 4 to 8 injections of toxoid.[2, 3] In our series of 38 cases, the titers of 19 were increased 5- to 10-fold; the titers of 31 were increased 2- to 10-fold, and the titers of 7 were increased 12 to 34 times. In every instance, however, the number of injections of toxoid necessary to raise the titer of sera to their maximum was greater than has been reported by others, the average number being about 13. The maximal titer obtained in the series was 28.8 I.U. This titer was obtained in the sera of 2 patients, and represented an increase of 4.5 and 7 times the initial titers.

It appears that certain individuals are better able than others to respond to the antigenic stimulus of infection or of artificially introduced toxoid. This is shown by the fact that those sera which reached the highest titers (10 units or more) attained their maximal levels in a definitely shorter time than those whose maxima were lower (4.4 units or less); the former group required an average of 44 days, while sera in the latter group reached their maximal level in about 71 days. In addition, most of the sera in the former group had initial titers above the normal range.

It has been previously observed that the titers obtained following toxoid-therapy do not remain maximal, but drop to a level which is usually higher than the initial titers.[2, 3] We found a similar drop in titers. This occurred about 1½-2 months after the cessation of toxoid-therapy in 29 individuals, and during the course of injections in 9. In the latter group the titer dropped and did not again rise in spite of continued injections of toxoid. In the entire group, the titers of 4 patients dropped to a level lower than the initial titers; the titers of 2 dropped to a level equal to the initial titers; and the titers of the remaining 32 dropped to a level higher than the original titers. The level finally reached in the majority of instances was from 2 to 8 times higher than the initial titer. We found no relationship between the maximal titers obtained and the final levels to which they fell. Neither was there any relationship between the extent of the rise of titers and the final levels. We have found that in general the higher titers fell somewhat more precipitously than the lower titers.

The authors submitted themselves to a course of administration of toxoid entirely comparable to an average course received by the patients. Their initial titers, which were 0.18 and 0.55 I.U., increased 4 and 6 times, respectively. Three months after the administration of toxoid had been stopped, the titer of one was still at the maximal level of 4 times the initial titer, while the titer of the other had dropped to a level 4 times the initial titer.

A detailed description of the clinical results following staphylococcal toxoid-therapy is to be made by Dr. Buchman. Briefly, it may be stated that the serologic response of the patients was not paralleled by a corresponding improvement in their clinical condition.

Summary. Following the administration of staphylococcal toxoid to a group of 38 patients with chronic staphylococcal osteomyelitis there was a definite increase in the staphylococcal antihemolytic titer, ranging from 2 to 34 times the initial titer. The titers fell, after a varying interval, from the maximum to a level which was generally higher than the initial titer. The definite increase in antihemolysin titer was not paralleled by a corresponding improvement in the patients' clinical condition.

8732 P

Ascorbic Acid Metabolism in Tuberculosis.

FRED H. HEISE AND GUSTAV J. MARTIN. (Introduced by L. U. Gardner.)

From the Trudeau Research and Clinical Laboratory, Trudeau, N. Y.

The literature relating to experimental animals indicates decreased resistance to tuberculous infection in animals on vitamin C deficient diets.[1-5] Conversely, animals with chronic disease are more easily precipitated into acute scurvy.[4] Clinical reports[1, 6, 7, 8] have appeared to the effect that scurvy renders a patient increasingly susceptible to tuberculosis, while the addition of vitamin C to the diet improves the prognosis in these cases. With the development of knowledge concerning subclinical vitamin deficiencies[9, 10] and of a method of judging the state of ascorbic acid nutrition in the human,[10] the study of this substance in its relation to tuberculosis was indicated.

Schroeder[11] has reported an increased requirement of vitamin C in tuberculosis. Three cases were studied: 2 for a period of 5 days during which they were fed 300 mg. of ascorbic acid daily. The report of Bullowa, *et al.*,[12] shows in one tuberculous patient a reduced excretion of vitamin C but with prolonged dosage of ascorbic acid a high peak was reached which fell rapidly on discontinuing the treatment.

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[1] Hojer, J. A., *Act. Pediat.*, 1924, **3**, supplement.

[2] Coulard, E., *Presse med.*, 1923, **31**, 611.

[3] Heymann, B., *Klin. Wchnschr.*, 1926, **5**, 59.

[4] Bieling, R., *Deutsche med. Wchnschr.*, 1927, **53**, 182.

[5] Mouriquand, G., Rochaux, A., Dosdet, L., *C. rend. de Soc. de Biol.*, 1925, **93**, 901.

[6] Hojer, J. A., and Westin, G., *Dental Cosmos*, 1925, **67**, 1.

[7] Nassau, E., and Singer, M. J., *Jahrb. f. Kinderheilk.*, 1922, **98**, 44.

[8] Von Niedner, *Med. Klinik.*, 1918, **14**, 333.

[9] Göthlin, G. F., *Nature*, 1934, **134**, 569.

[10] Harris, L. J., and Ray, S. N., *The Lancet*, 1935, **1**, 71; Abbasy, M. A., Harris, L. J., Ray, S. N., and Marrack, J. R., *The Lancet*, 1935, **2**, 1399.

[11] Schroeder, Hermann, *Klin. Wchnschr.*, 1935, **14**, 484.

[12] Bullowa, J. G., Rothstein, I. A., Ratish, H. D., and Harde, E., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 1.

The present study includes 44 cases with minimal, moderately advanced and far advanced tuberculosis; with every stage of activity under each type as shown by X-ray, symptomatology, and sedimentation rates.

A consideration of the daily excretion of vitamin C of patients with no complications, on the sanatorium diet, permits a rough classification into 4 groups: those excreting from 0 to 5 mg. of ascorbic acid daily; those from 5 to 8 mg.; those from 8 to 14 mg., and a fourth group comprising those excreting over 14 mg. daily. The method developed by Tillmans, *et al.*,[13] and Harris and Ray[14] for the estimation of vitamin C excretion in urine by titration against phenolindophenol was used throughout. Twenty-four-hour samples were collected, using glacial acetic acid as a preservative. In the group from 0 to 5 mg. daily excretion, there are 6 cases, 5 of which have active tuberculosis and one with questionable activity. This group consists of moderately and far advanced cases. The second group, 5 to 8 mg. daily excretion, has 14 cases, 5 of which are inactive and 9, or 70%, active. The group from 8 to 14 mg. comprises 21 cases with 7 active and 14, or 66%, inactive. The group 14 mg. or above contains 13 cases, one of which is active and 12, or 93%, inactive. This shows a rough parallelism between activity in uncomplicated tuberculosis and the daily excretion of ascorbic acid on a controlled diet. The factor of complications of the disease must be emphasized as it is realized that such conditions as acute sinusitis, etc., might render the determination valueless as an indication of the activity of the tuberculous process.

The second method of classification, namely, the response to a 4 oz. orange juice supplement to the diet (4 oz. of orange juice containing 55 mg. of ascorbic acid as determined by titration with phenolindophenol) offers a more accurate division according to activity. Two groups are here considered, one giving a positive response, the other a negative. A positive response is considered as an increase in excretion of ascorbic acid above 16 mg. daily within 2 weeks after the daily dose of 4 oz. of orange juice is instituted. The negative group here comprises 10 cases of which 80% are active. The positive group comprises 14 cases of which 100% are inactive. Again, it is to be emphasized that this report deals with uncomplicated disease.

The amount of ascorbic acid required daily by a normal healthy individual is from 15 to 20 mg. daily, whereas from our tests it would seem that between 55 and 138 mg. of ascorbic acid daily are required to bring a tuberculous patient into equilibrium as regards vitamin C nutrition. In one case dosages above 200 mg. daily did not cause a positive reaction.

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[13] Tillmans, J., Hirsch, P., and Hirsch, W., *Z. Untersuch. Lebens.*, 1932, **63**, 1.

[14] Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 303.

The investigation is being extended to demonstrate the requirements of vitamin C in tuberculosis and the possible prognostic value of determinations of the daily excretion of ascorbic acid. The present indications are that an increased requirement of ascorbic acid is manifested in tuberculosis and that the determination of the daily excretion seems to parallel determinations of activity made roentgenologically.

8733 P

Electric Potentials in Normal, Castrate, and Theelin Treated Rats.*

PHILIP V. ROGERS. (Introduced by E. Allen.)

From the Department of Anatomy, Yale University School of Medicine.

The development of the Burr-Lane vacuum tube microvoltmeter has opened up a new field in endocrine research. With the use of this machine a study of the electrical changes taking place during the reproductive cycle has been undertaken[1] since it has already been found that the instant of ovulation in the rabbit may be determined by the microvoltmeter.[2, 3] The following is a report of experiments with rats designed to record the electrical changes that take place during the normal oestrous cycle, and the effects of ovariectomy and of hormonal injections upon these changes.

In normal rats the difference in electrical potential between the vagina and the symphysis pubis changes greatly during the oestrous cycle. Daily readings taken while the animals were anesthetized with sodium amytal† have been completed on 35 female rats, from 2 to 8 months old, over periods varying from 10 to 30 days. In all cases vaginal smears were taken immediately before making the electrical readings in order to insure a close time correlation between the

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* Aided in part by a grant from the Fluid Research Funds of Yale, administered by Professor Edgar Allen.

[1] Rogers, P. V., *Anat. Rec.* (Suppl.), 1936, **64**, 40.

[2] Burr, H. S., Hill, R. T., and Allen, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 109.

[3] Hill, R. T., Allen, E., and Kramer, T. C., *Anat. Rec.*, 1935, **63**, 239.

† Supplied by the Eli Lilly Company.

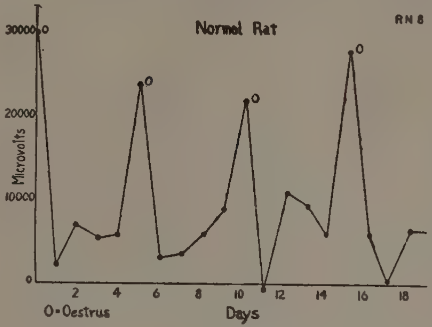

FIG. 1.

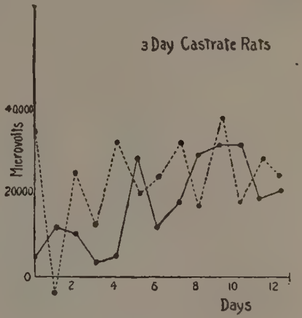

FIG. 2.

2 determinations. When these readings are plotted a very definite type of curve (Fig. 1) is obtained which is repeated during each cycle. When an animal is in oestrus, as shown by the cornified smear, the vagina is negative to the symphysis by several thousand microvolts. There then follows a sudden drop in the potential as the animal goes into postoestrus. In many cases at this time a reversal in polarity occurs so that for one reading the vagina is positive to the symphysis. During the period of dioestrus the potential remains low. As pro-oestrus approaches there is a gradual rise until the oestrous peak is once more attained. In 50% of the cases a lesser rise occurs 2 days before the animal comes into heat. Due to the fact that readings were taken only once a day this intermediate peak, occurring before pro-oestrus, may take place more often than recorded. This rise is not so great as that at oestrus, but is far above highest dioestrous records.

Removal of the ovaries results in an entirely different type of curve (Fig. 2). Instead of being cyclic in nature the daily readings vary in relation to each other so that the curve is quite patternless. The potential differences may be very great, even greater than in normal oestrus, rather than small as might be expected.

About 19 hours after injecting theelin‡ into castrate rats there is always a definite rise in the potential above the castrate level (Fig. 3). This peak seems to be comparable to the preoestrous rise that occurs in the normal rat. As in the normal animal, there is a drop before the rat comes into oestrus. When aqueous theelin is given (22 rats) the animal comes into oestrus during the third day, stays in for a short time, and then goes into postoestrus. During the period of cornification in these rats the electrical readings are high, forming a definite oestrous peak. This, as in the normal rat, is followed by a drop as the animal goes out of oestrus. When theelin

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‡ The aqueous theelin and theelin-in-oil were furnished by Parke, Davis & Co.

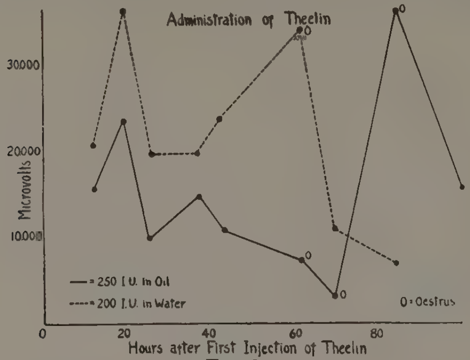

FIG. 3.

in oil is given the animals remain in heat for a longer time. These rats again show the rise about 19 hours after theelin injection but, in 8 out of 9 rats thus far studied, there is no second rise on the third day when they come into heat. The readings remain low until a few hours before postoestrus sets in when the expected rise in potential occurs. Further tests of these reactions are being made to ascertain if electric potentials are constant enough to furnish a unit of measurement for estrogenic substances.

Summary. In normal rats the difference in electrical potential between the symphysis pubis and the vagina is much greater at oestrus than during the rest of the cycle. Readings from castrate animals form a patternless type of curve. Administration of theelin causes two rises in potential: nineteen hours after the first injection, and just before the animal passes out of oestrus.

8734 P

Basal Metabolism in Experimental Anemias.

KUO-CHEN CH'EN AND HSIAO-CH'IEN CHANG.

From the Department of Medicine, Peiping Union Medical College, Peiping, China.

Basal metabolic changes have been observed in various types of anemia and in different stages of the same anemia. A number of theories have been advanced to explain this phenomenon, among which may be mentioned the hematopoietic activity[1] and the presence of immature cells in the blood.[2] While immature erythrocytes do consume more oxygen than normal red blood cells,[3] one can hardly expect them to appreciably influence the total metabolism since they constitute such a small fraction of the body mass. Baldridge and his associates[4-7] seem to have shown, in their recent investigation, a reciprocal relationship between the oxygen consumption and the nitrogen balance in various blood diseases but they, too, do not doubt the part sometimes played by the circulating reticulocytes in raising the respiratory metabolism.[4] The present study is planned to inquire into this particular question and to ascertain the behavior of the gaseous exchange in anemias produced by different methods.

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[1] Jeney, A., *J. Exp. Med.*, 1927, **46**, 689.

[2] Alt, H. L., *Arch. Int. Med.*, 1929, **43**, 488.

Young male adult albino rats of approximately the same age and weight (about 300 gm.) were used. Their normal oxygen consumption in the post-absorptive state was determined by repeated estimations according to the method of Davis and van Dyke.[8] Anemia was produced in one group of the animals by intraperitoneal injection of phenylhydrazine hydrochloride, 10 mg. per 100 gm. of body weight. In another group the rats were bled from the tail, about 2 cc. daily. Throughout the experimental period the following data were collected by standard methods: Body weight, oxygen consumption, body temperature, red and white blood cell counts, hemoglobin content, hematocrit reading, morphology of stained blood-smear, differential count and reticulocyte count. If the animal survived through the experiment it was sacrificed and autopsied.

All animals lost some weight in the course of the experiment, usually not exceeding 40 gm. The picture of the bone marrow is essentially the same in the 2 types of anemia, namely, marked hyperplasia involving both myelocytic and erythroblastic elements and nearly always some megaloblastic blood formation. In the first group only 4 animals were successfully studied, the majority succumbed too early to permit observation for a sufficiently long period. In them the phenylhydrazine produced a prompt drop of the red cell count and hemoglobin concentration. The reticulocyte count rose steadily, reached its maximum (varying between 45 and 50%) in from 5 to 8 days, and after being maintained at that level for about 2 days, declined slowly. Immediately following the injection of the drug the oxygen consumption showed a rise to from 10 to 30% above the control level but this rise only lasted for from 1 to 4 days. The peak of the reticulocyte curve lagged from 4 to 8 days behind the rise of metabolism. Leucocytosis and increase of the polymorphonuclear neutrophils accompanied the anemia.

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[3] Harrop, G. A., *Arch. Int. Med.*, 1919, **28**, 745.

[4] Baldridge, C. W., and Barer, A., *J. Clin. Invest.*, 1931, **10**, 529.

[5] Baldridge, C. W., and Barer, A., *Arch. Int. Med.*, 1933, **51**, 589.

[6] Barer, A., Paul, W. D., and Baldridge, C. W., *J. Clin. Invest.*, 1934, **13**, 15.

[7] Baldridge, C. W., *Arch. Int. Med.*, 1934, **54**, 517.

[8] Davis, J. E., and van Dyke, H. B., *J. Biol. Chem.*, 1932, **95**, 73.

In the second group in which the blood was depleted by bleeding, the red blood cell count and hemoglobin concentration dropped more slowly than in the preceding group but eventually reached just as low a level and remained there for a long time, as long as the bleeding was continued. A reticulocytosis of about 40% also took place in the course of from 10 to 12 days. The oxygen consumption, on the other hand, showed no significant change throughout the experimental period, nor after the discontinuation of bleeding. No marked leucocytosis occurred in this group.

In these experiments strict parallelism did not exist between the oxygen consumption and the reticulocyte count. In the phenylhydrazine anemia the increase in metabolism was fleeting in character and was manifest before the reticulocyte response reached its climax while in the post-hemorrhagic anemia the energy exchange showed no significant deviation from normal, during or after reticulocytosis. In the light of Baldridge's work the explanation of this difference seems evident. That is, in phenylhydrazine anemia there is an enormous destruction of the red blood cells to account for the transient increase in metabolism. In both conditions active hematopoiesis and high reticulocytosis are present but these changes *per se* do not seem to result in any marked alteration of the respiratory metabolism. This clearly shows that the metabolism of the immature blood cells contributes very little to the total oxygen consumption and that the basal metabolic changes in various blood diseases are dependent upon some other factor, such as increase of nitrogen catabolism as a result of blood cell destruction.

8735 C

Experimental Virus Infections in Chinese Hamster. II. Susceptibility to Street Rabies Virus.

ALBERT C. H. YEN. (Introduced by C. E. Lim.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

It has been noted[1] that the Chinese hamster is susceptible to fixed rabies virus, but its susceptibility to street rabies virus still remains to

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[1] Yen, A. C. H., PROC. SOC. EXP., BIOL. AND MED., 1936, **34**, 315.

TABLE I.
Showing Comparative Infectivity of Street Rabies Virus Introduced Through Different Routes.

Route of inoculation	Dilution of virus	No. of animals died/inoculated	Mortality in %	Incubation time, Days	Aver. incubation time, Days	Day of death	Aver. days of death
Intracerebral	1:4x10^2	13/13	100	10, 11, 11, 12, 12, 12, 12, 12, 12, 12, 13, 13, 14	12.0	11, 12, 12, 13, 13, 14,14, 14, 14, 14, 15, 17, 17	13.9
	1:1x10^4	10/10	100	12, 12, 12, 12, 12, 12, 12, 13, 13, 14	12.4	13, 14, 14, 15, 16, 16, 16, 17, 18, 19	15.8
	1:1x10^5	5/10	50	12, 13, 14, 14, 15	13.6	16, 16, 17, 17, 19	17.0
	1:1x10^6	2/10	20	14, 14	14.0	17, 18	17.5
Subcutaneous	1:10	4/10	40	15, 18, 20, 20	18.3	18, 23, 23, 24	22.0
	1:1x10^2	0/10	0	—	—	—	—
Intramuscular	1:10	6/10	60	14, 15, 15, 16, 16, 16	15.3	17, 19, 20, 21, 21, 23	20.2
	1:1x10^2	2/10	20	18, 20	19.0	28, 30	29.0
Intraperitoneal	1:10	1/10	10	14	14.0	19	19.0
	1:1x10^2	0/10	0	—	—	—	—

be determined. Observations on its behavior to infection by a strain of street rabies virus are, therefore, described here.

A fresh local strain of street rabies virus from dog was used in the present study. The original virus preserved in 50% glycerine was thoroughly ground in a sterile mortar and suspended in saline solution for inoculation. Chinese hamsters weighing 20-30 gm. were divided into several groups and each was separately inoculated with various dilutions of the dog brain virus through different routes as indicated in the table. The technique of inoculation was similar to that used previously.[1] The records of observations made on the incubation period, mortality, and days of death in relation to routes of inoculation and concentration of virus introduced are summarized in Table I.

Ten to 20 days after inoculation with the virus, the infected animals began to show increased excitability, which became very pronounced within 24 hours after its first appearance. The animals generally ran about constantly and bit the wire mesh of the cage. The movements of the body were shaky and unsteady. Fits of convulsion, especially when the animals were irritated, soon followed. Feedings were poorly taken and emaciation became noticeable. After 2 or 3 days of excitement, weakness or paralysis of the hind legs usually could be noted. In some animals, however, paralysis was observed to start from the front limbs. The animals usually died in 2 to 5 days after showing the first symptoms of excitement. When the course is acute as in animals inoculated intracerebrally with high concentration (1:400) of the virus suspension, death may result in 2-3 days after development of excitement without showing weakness of limbs.

The effectiveness of administration of the virus through different routes decreased in the following order: intracerebral, intramuscular, subcutaneous, and intraperitoneal. The first route was by far the most effective as fatal infection resulted with inoculation of virus suspension diluted as high as 1 to 1,000,000. The incubation time and day of death varied with two factors, namely the concentration of virus suspension and route of its introduction. When concentrated virus suspension was given, the incubation time and day of death tended to be shortened. They were also shortest when virus was introduced intracerebrally but were lengthened by 2 to 12 days when virus was introduced through other routes.

In brains of animals showing symptoms of the infection, Negri bodies were regularly found in the neurone cells of Ammon's horn and Purkinje cells of the cerebellum. The method used for the preparation of the sections was the same as described before.[1] The size of the Negri bodies varies from 3x4μ to 6x8μ. Not infrequently, 2 or 3 Negri bodies were seen in the cytoplasm of one neurone cell.

It is of interest to note that, from brains of hamsters dead of the infection, the virus (hamster passage virus) could be recovered, which was still infective for rabbits and white mice. But in contrast to the high infectivity of the original dog brain virus, the hamster passage virus, when inoculated intracerebrally in normal hamsters in concentration of 1:400, caused deaths of only 3 of 10 animals inoculated. These 3 animals died within 18-22 days with typical symptoms. The remaining 7 animals remained alive for over 5 weeks without showing any symptoms. Examination of the brains of the three dead animals and of three of the surviving animals killed by ether 4 weeks after inoculation of the virus, showed the presence of numerous Negri bodies only in the animals that died.

8736 C

Distribution of Precipitin in Serum Globulins of Different Species of Animals.

BACON F. CHOW. (Introduced by Hsien Wu.)

From the Department of Biochemistry, Peiping Union Medical College, Peiping, China.

Through the extensive investigations in different laboratories the antibodies in antipneumococcus horse serum (Type I) have been isolated in a relatively pure form,[1] and the chemical groupings responsible for the biological activities have been determined. Comparatively few studies have been made on the corresponding type of rabbit immune serum.

That immune rabbit and horse sera possess different immunological properties is well known, and their distinguishing characteristics have been summarized.[2] It is therefore of interest to find out whether the antibody is similarly distributed in the pseudo- and euglobulin fractions of the two sera. Thus globulins from the 2 immune sera, which contain all the antibody were fractionated into pseudo- and euglobulin portions. The amount and the biological activity, as measured by the maximum amount of protein precipitable by the homologous polysaccharide,[3] were quantitatively determined[4] for each fraction. A horse and several rabbits were immunized in the usual manner with the same strain of heat-killed pneumococci (Type I) and finally with live cultures. Freshly prepared sera from the 2 species of animals were used for the fol-

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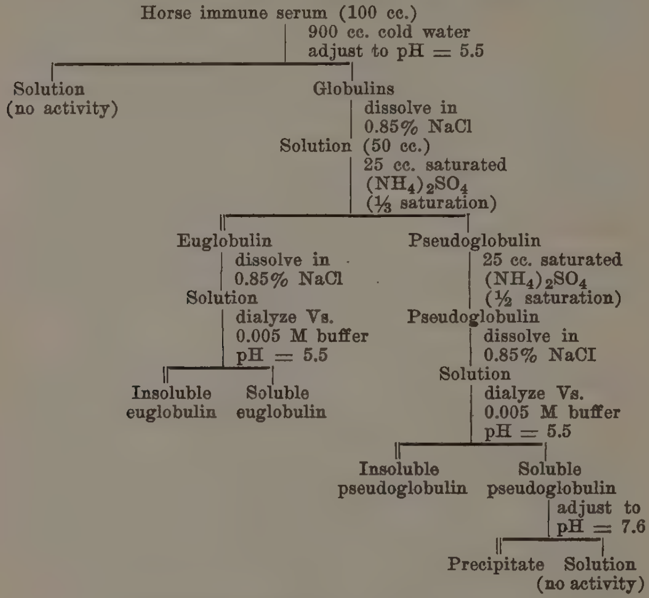
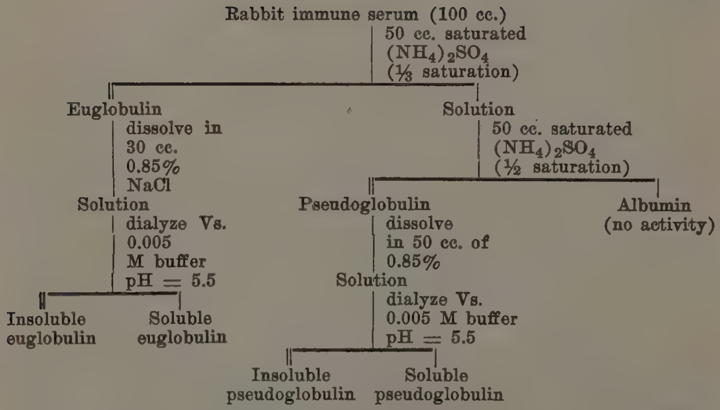
[1] Chow, B. F., and Goebel, W. F., *J. Exp. Med.*, 1935, **62**, 179.

[2] Horsfall, F. L., and Goodner, K., *J. Exp. Med.*, 1935, **62**, 485.





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[3] Chow, B. F., PROC. SOC. EXP. BIOL. AND MED., 1935.

[4] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1929, **50**, 809.

lowing experiments. Felton[5] has shown that practically all antibodies of antipneumococcus Type I immune horse serum are precipitated by a 10-fold dilution with water. This globulin fraction was therefore used as starting material for fractionation with ammonium sulfate. In the case of immune rabbit serum, only a small portion of the total globulin was precipitated with a 10-fold dilution with water. Unlike the immune horse serum, the precipitable globulin contained no activity. The eu- and pseudoglobulin fractions were obtained directly from the immune serum at ⅓ and ½ saturation of ammonium sulfate, respectively. The schemes of fractionation are shown on the preceding page.

The relative quantity and the maximum amount of protein precipitable by the homologous polysaccharide in each fraction are shown in Table I.

TABLE I.

	Horse immune serum		Rabbit immune serum	
	% of the total serum protein	% of protein precipitable by the polysaccharide	% of the total serum protein	% of protein precipitable by the polysaccharide
Insoluble euglobulin	3.0	10.0	8.3	16.5
Soluble euglobulin	1.5	11.5	11.9	29.6
Insoluble pseudoglobulin	2.0	20.0	9.1	5.0
Soluble pseudoglobulin	8.0	65.0	10.2	26.0

The results of this comparative study bring out two striking differences between the immune sera of the two species of animals. The first is that in horse serum the antibody in pseudoglobulin fraction soluble in a buffer (0.005 M) at pH = 5.5 is completely precipitated when the pH of the solution is adjusted to 7.6. Whereas from a corresponding fraction of the rabbit immune serum no trace of precipitate is obtained. The second difference is that in the case of immune horse serum, the main part of the antibody is concentrated in the pseudoglobulin fraction, whereas the opposite is true with the immune rabbit serum, that is, the euglobulin fractions contain the major portion of the antibody.

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[5] Felton, L. D., *Boston Med. and Surg. J.*, 1924, **190**, 815; *J. Infect. Dis.*, 1925, **37**, 199, 309.

8737 P

The Incidence of the Newer Types (Cooper) of Pneumococcus in Peiping.

C. J. Wu and S. H. Zia.

From the Department of Public Health and Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

In a previous study on pneumococcal lobar pneumonia as occurring in Peiping[1] it was pointed out that the distribution of the various types of pneumococci according to the old classification compared closely with the experience in America. Recently, Cooper and her associates[2] by the use of specific agglutinative tests have separated those strains of pneumococcus hitherto designated as Group IV into 29 types and have produced specific antisera for a few of these types for therapeutic use. This finding has been substantiated by other workers and Group IV strains isolated from normal people as well as from pneumonic patients have been so classified.

Since January, 1935, we have been interested in the type-distribution of pneumococci isolated from routine cultures made of patients with a variety of diseases and have been able to classify a number of the Group IV strains. We are indebted to Miss Cooper who kindly supplied us with diagnostic sera.

Though it is impossible, on account of the small number tested, to make any generalization concerning the preponderance or scarcity of any one particular type, the distribution corresponds well with that found by other workers. In addition, a few more points of interest may be mentioned. Of the 22 cases of lobar pneumonia in adults 8 were due to Type V and 5 to Type IX. Eleven of these patients had bacteremia and 3 empyema; 8 died. Bronchopneumonia as a terminal complication in adult patients is found to be caused by a great variety of types. Pneumonias caused by these newer types is a very grave condition in children. The majority of our patients died. Of particular interest is the fact that 7 of the 22 cases in this series were caused by Type XIV, whose importance as a cause of pneumonia in children has been pointed out by Nemir,[3] Bullowa[4] and others. These authors also demonstrated the efficacy

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[1] Wu, C. J., and Chiu, T. Y., *Chinese M. J.*, 1933, **47**, 545.

[2] Cooper, G., Rosentein, C., Walter, A., and Leizer, A., *J. Exp. Med.*, 1929, **49**, 461; *ibid.*, 1932, **55**, 531.

[3] Nemir, R. L., *J. Ped.*, 1934, **3**, 827.

[4] Bullowa, J. G., *J. Clin. Invest.*, 1935, **14**, 273.

TABLE I.
Types (Cooper) of Pneumococcus as Encountered in Peiping.

Type	All cases	Lobar pneumonia	Broncho-pneumonia	Pneumonia, children
IV	4		1	
V	15	8	1	2
VI (a, b)	18			3
VII	2	1	1	
VIII	10		1	
IX	10	5	1	1
X	12	1	2	
XI	8		2	2
XII	4	1		
XIII	7			1
XIV	11	2		7
XV	5	1		2
XVI	4		2	
XVII	3			
XVIII	4			
XIX	9	2		
XX	5		1	1
XXI	2			1
XXII	3			
XXIII	14	1	2	1
XXIV	2		1	
XXV	1		1	
XXVII	1			1
XXVIII	1			
XXIX	2			
XXX	0			
XXXI	1			
XXXII	0			
Unclassified	4			
Total	162	22	16	22

of treatment of such cases with the specific Type XIV antiserum. The especial virulence of Type XIV for young children and babies was further borne out by the fact that 5 of our 7 cases developed purulent meningitis due to the same type, 6 had bacteremia and all cases ended fatally. Four strains with typical cultural characteristics and virulence for mice failed to be agglutinated by any of the sera used. These are now being further investigated.

8738 P

Antigenicity of Diphtheric Toxin Subjected to Photodynamic Action of Methylene Blue.

F. C. Lin. (Introduced by S. H. Zia.)

From the Department of Bacteriology and Immunology, Peiping Union Medical College, Peiping, China.

Although the inactivating effect of methylene blue in the presence of light on bacteriophage, viruses, toxins and bacteria has been generally agreed upon, the antigenicity of substances so treated is still disputed. Some reports have been positive,[1] others, negative.[2] Our observations indicate that definite antigenic property is possessed by photodynamically inactivated diphtheric toxin.

A single batch of toxin containing 400 M.L.D. per cc. (original titre 2000 M.L.D. per cc.) and one of toxoid (20 Lf. per cc.) obtained from the National Epidemic Prevention Bureau were used in this study. Before exposure to the photodynamic action of methylene blue (concentration of dye $4\text{x}10^{-5}$) in sunlight, the toxin was diluted 1:25 with sterile saline; when electric light[3] was used, the dilution was 1:100. Normal guinea pigs of approximately the same weight were divided into 3 groups of 7 each. All animals of the first group received 8 biweekly subcutaneous injections of 5 cc. of diluted detoxified toxin subjected to the action of sunlight and dye; those of the second group, 8 injections of 5 cc. of diluted detoxified toxin treated with electric light and dye; those of the third group, 8 injections of 0.2 cc. toxoid. One month after the last injection, different amounts of diphtheric toxin were given to all animals in these 3 groups. It was found that the animals of the first and second groups could withstand the toxin up to 640*-960 M.L.D, while those of the third up to 160-320 M.L.D. It is obvious that the toxin after treatment with light and dye still possessed the power of provoking active immunity to a degree even higher than that produced by injections of toxoid.

The production of circulating antitoxin was studied in 6 rabbits divided in 3 groups. Each group was given injections either of sunlight- and dye-treated toxin, electric light- and dye-treated toxin or of toxoid in doses and intervals similar to those described above for guinea pigs. Ten days after the last injection it was found that the average amount of toxin that could be neutralized by 1 cc. of serum in 30 minutes at room temperature, was 10 M.L.D. for the animals of the first group, 25 M.L.D. for those of the second group, and 4 M.L.D. for those of the third group.

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[1] Perdrau, J. R., and Todd, C., *J. Comp. Path. and Ther.*, 1933, **46**, 76; Galloway, I. A., *Brit. J. Exp. Path.*, 1934, **15**, 97.

[2] Shortt, H. E., and Brooks, A. G., *Indian J. M. Res.*, 1935, **22**, 557; Shortt, H. E., and Mallick, S. M. K., *Indian J. M. Res.*, 1935, **22**, 529; Lippert, K. M., *J. Immun.*, 1935, **28**, 193.

[3] Lin, F. C., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 337.

* M.L.D. was calculated according to the individual body-weight.

Traces of residual toxin in the photodynamically treated material were demonstrated by the development of late paralysis in some guinea pigs after having received repeated large doses of the treated toxin. The possibility of this residual toxin being partially responsible for the antigenicity of the detoxified toxin can not be excluded. But in view of the high immunity produced the result is comparable to that obtained by the use of toxoid with the addition of sublethal doses of active toxin.

8739 P

Comparison of the Antigenicity of Three Forms of Staphylococcal Toxoid.

KEH-HUNG LI. (Introduced by Richard H. P. Sia.)

From the Department of Medicine and the Department of Public Health, Peiping Union Medical College, Peiping.

In the preceding report it was shown that staphylococcal toxin after complete detoxification by the photodynamic action of methylene blue still retains its antigenic property. This note records the results of a preliminary study of the antigenic value of this "methylene blue toxoid" in comparison with 2 other common forms.

Materials and methods. (a) A single lot of toxin, No. 9669F, was used for the preparation of the 3 forms of toxoid. Formol-toxoid was prepared with 0.3% formalin. The alum-precipitated formol-toxoid was made by the method of Wells, Graham and Havens.[1] A third portion of toxin was treated with methylene blue, 1:50,000, and exposed to direct sunlight for 3 hours. The 3 toxoids did not hemolyze rabbit-erythrocytes. Immunization and estimation of antigenic response were done as described in the preceding paper.

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[1] Wells, Graham and Havens, *Am. J. Pub. Health*, 1932, **22**, 648.

At each stage the procedures were carried out simultaneously and in identical manner.

TABLE I.

Subcutaneous injections in 0.2 cc. doses	No. of rabbit	Staphylococcal Antitoxin in Sera (Provisional units per cc.)				Local Reaction	Dermo-necrotic doses resisted
		End of first week	End of second week	End of third week	End of fourth week		
I. Formol-toxoid							
a. Single dose	74	>0.4	>0.4	>0.4	0.8	+	6.2
	7	>0.4	>0.4	>0.4	>0.4	+	1.6
b. Weekly doses	39	>0.4	1.6	—	—	+	—
	28	>0.4	0.4	16.0	6.4	±	>25
II. Alum ppt. td.							
a. Single dose	67	0.4	1.6	0.8	0.4	++	1.6
	88	3.2	3.2	0.8	1.6	+++	3.1
b. Weekly doses	3	>0.4	1.6	8.0	6.4	++	25–
	44	>0.4	0.8	8.0	3.2	+++	25
III. M.B. toxoid							
a. Single dose	86	>0.4	>0.4	>0.4	>0.4	0	3.1
	92	>0.4	0.8	0.8	>0.4	±	3.1–
b. Weekly doses	4	>0.4	>0.4	6.4	3.2	±	6.2
	77	>0.4	>0.4	3.2	1.6	0	12.5

For local reaction:

0	indicates	absence of any apparent reaction.
±	”	slight redness for 1 day.
+	”	definite redness for more than 1 day.
++	”	redness and induration for 3 days.
+++	”	redness, induration and fluctuation.

The results are summarized in Table I. The small number of animals used does not allow a final conclusion. The results suggest that (A) the M. B. toxoid is about as effective as the formol- and alum-precipitated toxoids, and (B) the M. B. toxoid seems to cause less local reaction than the other forms of staphylococcus toxoid studied.

8740 P

Antigenicity of Staphylococcal Toxin Detoxified by the Photodynamic Action of Methylene Blue.

Keh-Hung Li. (Introduced by Richard H. P. Sia.)

From the Department of Medicine and the Department of Public Health, Peiping Union Medical College, Peiping.

The fact that some organisms, viruses, bacteriophages and toxins may be rendered harmless and inactive by the photodynamic action of methylene blue has been well established. Since Perdrau and Todd[1] reported that their virus of canine distemper so inactivated possessed a high antigenic property, the antigenic value of these products has become an interesting problem. Rivers[2] cautioned workers on the general principle that when the degree of inactivation could not be accurately measured one should be careful in attributing antigenicity to these so-called inactivated agents. Since titrations of minute residual amounts of active virus are still impossible, one naturally turns to bacterial toxins for an answer to this question. Lippert[3] reported failure to demonstrate antigenicity in his inactivated tetanus-toxin. Lin[4] could not completely detoxify diphtheric toxin. In this note, we present the following findings: Staphylococcal toxin can be completely detoxified by the photodynamic action of methylene blue and when so detoxified still possesses antigenic value.

The method of preparing toxins was a modified combination of those recommended by Parker and Dolman.[5] The medium was 0.3% agar with 4% Witte's peptone in beef broth buffered at pH 7.0. After incubation in an atmosphere containing about 20% carbon dioxide, it was filtered through cheesecloth, paper and Berkefeld candles. The final filtrate was our toxin. Only those preparations whose potency equalled or surpassed the minimal standards outlined by Dolman,[7] were employed. Gruebler's "Methylenblau" was used for detoxification.

To measured volumes of toxin, minute quantities of the stock dye-solution were added and thoroughly mixed. These mixtures, contained in large covered petri dishes, were placed on ice 20 cm. below a 100 watt lamp. At appropriate intervals, samples of these exposed mixtures and of control mixtures kept in the dark were titrated for hemolysis of rabbit-erythrocytes, dermo-necrosis and killing effect in rabbits.

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[1] Perdrau and Todd, *J. Comp. Path. and Therap.*, 1933, **46**, 78.

[2] Rivers, *Am. J. Med. Sc.*, 1935, **190**, 435.

[3] Lippert, *J. Immunol.*, 1935, **28**, 193.

[4] Lin, Proc. Soc. Exp. Biol. and Med.

[5] Parker, *J. Exp. Med.*, 1924, **40**, 761; Dolman, *J. Infect. Dis.*, 1934, **55**, 172.

[7] Dolman and Kitching, *J. Path. and Bact.*, 1935, **41**, 137.

The completely detoxified product was given to rabbits with known natural antitoxin-titres. At intervals during immunization the animals' sera were titrated for neutralizing property according to the hemolytic method of Hartley and Smith.[6] At the end of the immunizing injections the animals were tested for their cutaneous resistance to staphylococcal toxin according to the method of Dolman.[7]

Results. The photodynamic action of methylene blue can completely remove all the 3 known toxic properties of staphylococcal toxin. Table I illustrates the effect upon the hemolytic property.

TABLE I.
Maximal Dilution of Staphylococcal Toxin which Completely Hemolyzes 1% Rabbit-erythrocytes after 1 hr. in Waterbath at 37° C.
(Undiluted toxin 9669I, pH 7.2, exposed to 100-watt Mazda light at 20 cm.)

Hours Exposed	Concentration of methylene blue		
	1:10,000	1:50,000	1:500,000
To light			
1	1/1024+	1/1024+	1/1024+
2	1/512	1/1024	1/1024
4	1/256	1/256	1/1024
6	1/32	1/64	1/512
8	None	1/2	1/32
In the dark			
72	1/1024+	1/1024+	1/1024+
240	1/1024+	1/1024+	1/1024+

TABLE II.
Response of Rabbits to Completely Detoxified of Staphylococcal Toxin 9669I.

		Staphylococcal Antitoxin in Sera (International Standard Units per cc.)				
Mode, am't injected	Rabbit No.	Initial titration	1 week after 1st injection	1 week after 2d injection	2 weeks after 2d injection	Dermo-necrotic doses resisted
A. Intravenously, 5 cc.						
	1	>0.05	0.05	0.80	3.20	6.25
	2	0.05	>0.05	Died 3 days after 2d inj.	—	—
	3	0.05	>0.05	0.80	6.420	6.25
B. Subcutaneously, 0.2 cc.						
	4	>0.05	0.40	6.40	25.6	25
	5	0.10	>0.05	3.20	12.8	6.25
	6	0.10	>0.05	0.05	3.20	6.25

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[6] Hartley and Smith, League of Nations: *Quart. Bull. of Health Org.*, Special No. Jan., 1935.

It was also found that originally potent toxins became non-skin-necrotizing when injected endermally in undiluted form and non-lethal to rabbits when given intravenously in doses of 3 cc. per kilogram body weight.

Table II shows a good antigenic response in rabbits following intravenous or subcutaneous injections of staphylococcus toxin detoxified by the photodynamic action of methylene blue.

8741 C

Negative Effect on Blood of Normal Rabbits of Inoculation of Killed *Leishmania donovani.*

CHIA-TUNG TENG. (Introduced by Claude E. Forkner.)

From the Department of Medicine, Peiping Union Medical College, Peiping, China.

Leucopenia is one of the outstanding features in kala-azar. It develops early in the course of the disease and is frequently out of proportion to the anemia. This fact has been well recognized, and its diagnostic value repeatedly emphasized. The mechanism of the development of this phenomenon is, however, by no means understood. By incubating leucocytes with blood serum, Maggiore and Sindoni[1] claimed to have demonstrated the presence in the blood serum of patients with visceral leishmaniasis, of a substance which is leucocytolytic. Arena[2] confirmed this finding. Their results were not at all conclusive, and there has been no further confirmation. Some workers[3, 4] believed that leucopenia as well as anemia in this disease is myelophthisic, the overgrowth of the macrophages ("clasmatocyte tissue") being comparable to the metastatic growths of carcinoma in blood-forming organs. This does not explain satisfactorily the early appearance of leucopenia when the infection is only beginning and the "crowding out" of the myeloid tissue hardly can have taken place.

It has been suggested,[5, 6, 7] in discussing the problem of leucopenia in general, that there are possibly 2 factors concerned in the maintenance of a normal level of leucocytes in the peripheral blood, *viz.*, a maturation factor and a chemotatic factor. Whether or not such factors also operate in the production of leucopenia in kala-azar, one can only conjecture. Forkner and Zia[8] have shown that pentose-nucleotide, which was considered by Doan[5] as an example of a chemotatic substance for leucocytes, did not produce any significant change in the number of leucocytes when administered intramuscularly to patients with kala-azar. One may further postulate that there may be present in this disease an unknown factor which has a depressive effect on the myeloid tissue. The fact that the leucopenia and anemia begin to improve quite steadily soon after the institution of specific treatment may suggest the actual presence of some such factor which can be removed or counteracted by controlling the infection. It seems, therefore, worthwhile to investigate whether such a factor is present in the body of *Leishmania donovani* or its metabolites. It occurred to us that, by inoculation into animals of the killed organisms or their metabolites, it might be possible to bring about in the blood a picture comparable to that produced by actual infection.

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[1] Maggiore, S., and Sindoni, M., *Pediatrica,* 1917, **25**, 81.

[2] Arena, G., *Pediatrica,* 1927, **35**, 465.

[3] Hu, C. H., and Cash, J. R., *Trans. Far East. Assn. Trop. Med., 7th Congress, British India,* 1927, **3**, 62.

[4] Keefer, C. S., Khaw, O. K., and Yang, C. S., *Nat. Med. J. China,* 1929, **15**, 731.

[5] Doan, C. A., *J. A. M. A.,* 1932, **99**, 194.

[6] Beck, R. C., *Arch. Int. Med.,* 1933, **52**, 239.

[7] Beck, R. C., *Am. J. Path.,* 1934, **4**, 189.

Fresh cultures of *Leishmania donovani* were obtained from a puncture of the spleen of a patient with kala-azar. They were subcultured in a number of tubes containing Novy-MacNeal-Nicolle (N.N.N.) medium at a temperature of from 22 to 23°C. for from 2 to 4 weeks, at the end of which all the tubes invariably contained rich growths of the flagellates. The content of each tube was examined for the presence of bacteria and was discarded if contamination was present. The fluid portion of these tubes containing numerous flagellates in pure culture was then pooled. Tricresol was added in an amount equivalent to 0.5%, which caused the fluid to change its color from dark red to brown and the formation of brownish sediments. All the flagellates were killed by this treatment, as was demonstrated by the fact that they became unrecognizable under the microscope and could no longer be cultured in N.N.N. medium. The amount of the sediment in this suspension apparently depended on the richness of the culture, and it varied from 7 to 10% of the total volume as determined by centrifuging in a hematocrit tube. The suspension was kept in rubber-stoppered bottles in an ice chest. Immediately before use, it was thoroughly mixed.

Six normal rabbits, each weighing from 1,500 to 2,000 gm., were taken from stock without selection. They were kept in separate cages. Two of these animals (R.19 and R.20) were kept as controls, to which no suspension of *Leishmania donovani* was given. Two other animals (R.21 and R.22) received the suspension intraperitoneally in a short course of intensive treatment. The remaining 2 animals (R.23 and R.24) were given daily subcutaneous injections of the suspension for 48 days and, in addition, a short course of intensive treatment with intraperitoneal injections as for R.21 and R.22.

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[8] Forkner, C. E., and Zia, L. S., unpublished data.

All animals were kept under the same environmental conditions, fed regularly at the same time each day and the studies were conducted at comparable periods throughout the experiment. All animals were allowed a period of control observations for 4½ months, during which time hemoglobin determinations, red blood corpuscle counts, white blood cell counts, and differential counts were made weekly from blood obtained by puncture of the ear vein. The body weight and temperature were recorded at the same time. During the period of continuous daily injections of the suspension in the case of R.23 and R.24, total white blood cell counts and differential counts were done daily, while the hemoglobin determinations and red blood corpuscle counts were made weekly. For the control animals, these observations were made once a week.

At the end of the experiment, all animals were killed by intravenous injections of air. The liver, spleen, lymph-nodes, lungs, kidneys, and the bone-marrow of the femur and the tibia were removed and fixed immediately in Zenker-formol solution (10% formalin). Sections were made and stained with hematoxylin and eosin. Comparative studies of the histologic pictures were made.

Results. 1. A slight decrease in the total leucocyte count and a relative decrease in the percentage of neutrophils occurred within 2 hours after the subcutaneous injection of the suspension of *Leishmania donovani.* Normal counts were restored about 3 hours after the injection. 2. There was no constant or significant change in the leucocyte and neutrophil counts in rabbits receiving either daily subcutaneous injections of relatively small amounts of the suspension or an intensive treatment with intraperitoneal injections of large amounts of it. (Chart 1. The findings in the case of rabbits Nos. 21, 22, and 23 were similar to those in rabbit No. 24.) 3. There was no decrease in the value of hemoglobin or in the number of red blood corpuscles following the injections of the suspension. The platelets remained normal in number. 4. The body weight and the temperature of the animals were not affected by the treatment. 5.

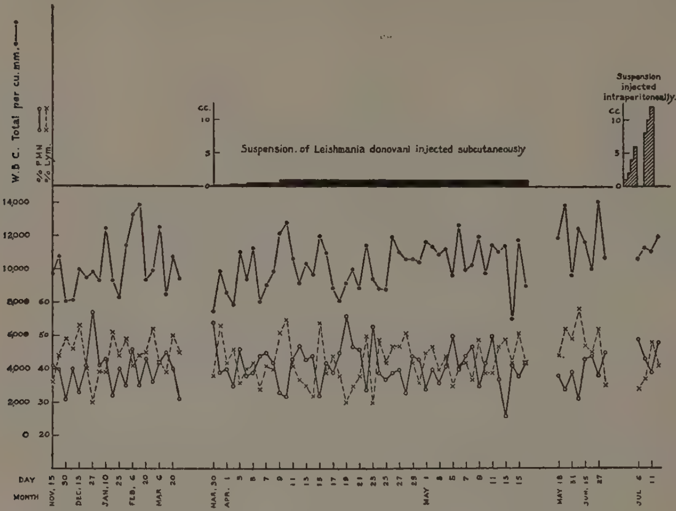

CHART 1.

Showing negative effect of parenteral inoculation of the suspension on the white blood cell counts of Rabbit No. 24 (R.24).

Studies of the histologic picture of the liver, spleen, lymph-nodes, lungs, kidneys, and the bone-marrow of the femur and the tibia did not reveal any striking difference in the animals treated as compared with those of the controls.

Rabbits were used in this experiment primarily because of the fact that they were more convenient for the study of changes in the blood. It is generally known that rabbits are relatively refractory to infection with *Leishmania donovani* and thus they may not be entirely suitable animals for this experiment. On the other hand, animals highly susceptible to the disease, such as Chinese hamsters *(Cricetulus griseus)*, are rather too small for repeated daily studies of the blood. It should be emphasized that what is true in rabbits might not be applicable to susceptible animals or human beings.

The suspension used for the injections was composed of killed flagellates of *Leishmania donovani* (not Leishman-Donovan bodies) in the fluid medium (N.N.N.) of the culture. It contained, therefore, both the substance of the organisms, the metabolites of them, and the medium. The physical nature of this suspension made it unsuitable for intravenous administration.

The decrease of leucocytes shortly following the injection of the suspension was only slight and transient. The same phenomenon has been observed by others following inoculations of bacterial vaccines.[9] It is probably of no great significance.

The mechanism of leucopenia in kala-azar remains an unsolved problem.

8742 P

Liberation of Acetylcholine from the Perfused Human Placenta.

HSI-CHUN CHANG.

From the Department of Physiology, Peiping Union Medical College, Peiping, China.

A fresh human placenta was washed by an intra-arterial saline infusion until the fluid coming from the placenta became colorless. The wet weight having been taken, a piece weighing about 30 gm. was removed and extracted for the basal acetylcholine (hereinafter designated as a.c.) content. The placenta was supported on wire gauze fixed in the middle part of a large funnel, and perfused with a known volume (445-510 cc.) of oxygenated saline by means of a Dale-Schuster pump at a pressure of 90-100 mm. Hg. The whole outfit was kept in a small room which could be readily maintained at body temperature. At hourly intervals, the placenta was detached, weighed and a small piece removed for extraction. The perfusion-fluid was measured and 2 cc. were taken out each time for titrating its a.c.-activity. All the samples of tissue were extracted for 2 hours with 2 volumes of 96% alcohol. The different extracts and the samples of the perfusion-fluid were assayed on the same *rectus*-preparation.[1] The influence of body-temperature, potassium, and eserine has been studied in 5 experiments with uniform results; potassium was suggested for investigation by Feldberg.[2] Fig. 1 illustrates the essential points obtained.

It is evident that the potassium increases a.c. in the perfusion-fluid at the expense of the tissue-a.c., while eserine and an increase of temperature to 36°C. increase a.c. in the tissue as well as in the fluid.

Potassium may be considered to cause a transfer of a.c. from

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[9] Wells, C. W., *J. Inf. Dis.*, 1917, **20**, 219.

[1] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[2] Personal communications.

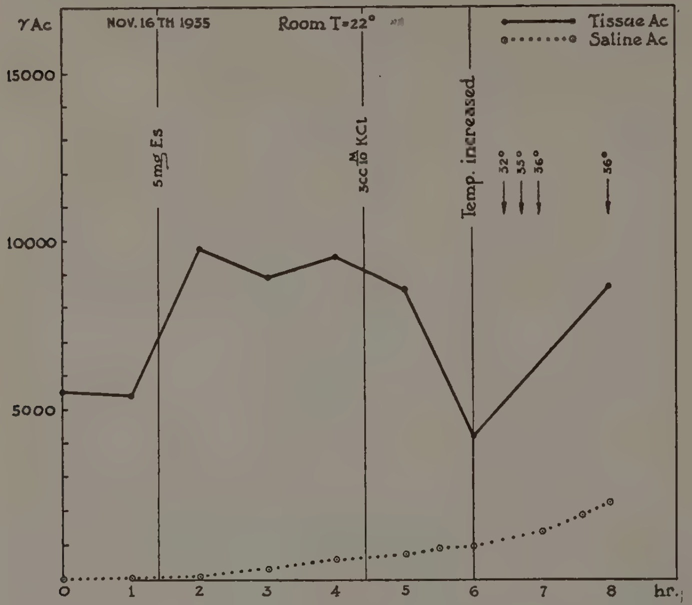

Fig. I

tissue to fluid, as Brown and Feldberg[3] have shown, whereas eserine and heat, confirming our previous experiments,[4] appear to stimulate a new formation of a.c. through some complex mechanism which is as yet not completely known. Mention may be made here that a.c.-genesis by eserine occurs also at 37°[4] and 3°C.[5]

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[3] Brown, G. L., and Feldberg, W., *J. Physiol.*, 1935, **84**, 127.

[4] Chang, H. C., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1001.

[5] Unpublished data.

8743 P

Immunologic Studies on New Preparation of Type-Specific Polysaccharide from Pneumococcus Type I.

Bacon F. Chow. (Introduced by Hsien Wu.)

From the Department of Biochemistry, Peiping Union Medical College, Peiping, China.

The isolation of chemically different polysaccharides by Heidelberger and Avery[1] from various types of pneumococci places the problem of type-specificity in immunologic reactions on a definite chemical basis. Since the polysaccharide from Type I pneumococcus, called the "Soluble Specific Substance," was not antigenic, it can only be considered as a hapten of the pneumococcal antigen. Later Wadsworth,[2] Enders,[3] and others obtained preparations of polysaccharide from pneumococcus Type I, which were antigenic in mice. Therefore they were believed to be different from the soluble specific substance.

Recently Avery and Goebel,[4] by omitting the alkaline treatment, isolated an acetyl polysaccharide from the autolyzed broth-culture of pneumococcus Type I. Like similar preparations from other laboratories, the acetyl polysaccharide was antigenic in mice. However, the antigenicity was destroyed by treatment with alkali with accompanying loss of acetyl groups. Thus it is probable that Enders' and Wadsworth's polysaccharides may be identical with the acetyl-polysaccharide. Enders and his colleagues[5] have already confirmed the observations of Avery and Goebel.

By using a method which minimized hydrolysis by acid or alkali in the preparation of polysaccharide, we have obtained a product even more complete than the acetyl polysaccharide. An autolyzed broth-culture was not autoclaved, as in Avery and Goebel's procedure, but was concentrated at a reduced pressure so that the temperature was never higher than 37°C. After the subsequent purification according to Avery and Goebel's method[4] a white polysaccharide was obtained. This polysaccharide reacted with a homologous immune-rabbit serum, previously absorbed with the acetyl polysaccharide. On the other hand, the acetyl polysaccharide did

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[1] Heidelberger, M., and Avery, O. T., *J. Exp. Med.*, 1924, **40**, 301.

[2] Wadsworth, A., and Brown, R., *J. Immunol.*, 1933, **24**, 349.

[3] Enders, J. F., *J. Exp. Med.*, 1932, **55**, 191.

[4] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

[5] Enders, J. F., and Wu, C. J., *J. Exp. Med.*, 1934, **60**, 127; Pappenheimer, A. M., Jr., and Enders, J. F., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 37.

TABLE I.

Precipitin-reaction of newly-prepared and acetyl polysaccharides of pneumococcus type I in homologous antiserum before and after absorption with the polysaccharides. The precipitin-reaction was performed by adding 0.5 cc. of polysaccharides of different dilutions to 0.5 cc. of diluted serum containing 2 cc. of original serum and 3 cc. of saline. The mixtures were incubated at 37° C. for 2 hours and chilled in the ice-box over-night.

Antipneumococcus rabbit serum (Type I)	Final dilutions of the newly-prepared polysaccharide								Final dilutions of the acetyl polysaccharide							
	1:1,000	1:10,000	1:40,000	1:100,000	1:200,000	1:400,000	1:800,000	1:2,000,000	1:1,000	1:10,000	1:40,000	1:100,000	1:200,000	1:400,000	1:800,000	1:2,000,000
Unabsorbed	++++*	++++*	++++	+++	++	+±	+	+	+++	++++*	++++	+++	++	+	+	±
Absorbed with acetyl polysaccharide	++++*	++++*	++++	++±	++	+	+	±	—	—	—	—	—	—	—	—
Absorbed with the newly prepared polysaccharide	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

*Disc formed.

not react with its homologous immune-rabbit serum absorbed by the new polysaccharide (Table I). A quantitative precipitin-titration showed that the new polysaccharide precipitated almost 3 times more antibody-protein than did the acetyl polysaccharide.

Although an antipneumococcal serum absorbed with our polysaccharide was still agglutinative and specifically protected mice from an otherwise fatal dose of pneumococci (Type I), the titre was greatly reduced.

The new polysaccharide in small doses produced active immunity in mice. Rats given intravenous injections of the polysaccharide-solution produced sera which protected mice from a fatal dose.

When the polysaccharide solution was heated at 100°C. in 0.05 N NaOH or 0.5 N acetic acid, the hydrolytic product still produced active immunity in mice, but failed to precipitate the immune serum absorbed with the acetyl polysaccharide.

It appears, therefore, that our polysaccharide may be the parent substance from which the acetyl polysaccharide could be obtained by appropriate treatment.

8744 P

Metabolism of Ethyl Esters of Fatty Acids.

H. J. DEUEL, JR., L. HALLMAN, J. S. BUTTS AND S. MURRAY.

From the Department of Biochemistry, University of Southern California School of Medicine, Los Angeles.

It has been demonstrated[1] that the conversion of caproic, butyric, and beta-hydroxy butyric acids to acetone bodies in fasting rats by beta oxidation is quantitative, whereas greater amounts of ketone bodies originate after sodium caprylate than after isomolecular quantities of sodium acetoacetate are fed. The latter phenomenon suggests that delta oxidation occurs in the latter case. No ketone bodies are formed when the sodium salts of the fatty acids with an odd carbon chain, as propionic, valeric, heptoic or nonylic acids were fed. It was later demonstrated[2] that the conversion of the odd chain fatty acids into glycogen must represent an approximately quantitative transformation by beta oxidation into propionic acid.

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[1] Butts, J. S., Cutler, C. H., Hallman, L., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1935, **109**, 597.

[2] Deuel, H. J., Jr., Butts, J. S., Hallman, L. F., and Cutler, C. H., *J. Biol. Chem.*, 1935, **112**, 15.

The fatty acids with an even number of carbon atoms were entirely ineffective as glycogen formers. However, it was impossible to feed the soaps of the fatty acids having a greater number of carbon atoms than 9 in an equimolecular dose to that found effective with the shorter chain fatty acids because of the relatively large quantities of solution which must be administered.

In the present experiments, by administering the fatty acids as their ethyl esters it has been possible to feed isomolecular quantities of all of the fatty acids up to stearic acid in similar doses to those employed in the earlier work—namely, 15 gm. per square meter of body surface per day, calculated as acetone.

The average excretion of acetone bodies in fasting male rats, second to fourth days, calculated as acetone in grams per square meter of body surface per day after feeding the ethyl esters was as follows: acetoacetate, 2.01 (57)*; butyrate, 1.37 (27); caproate, 1.95 (31); caprylate, 7.29 (12); caprate, 6.74 (16); laurate, 6.75 (17); myristate, 5.21 (9); palmitate, 2.94 (10); stearate, 2.40 (11); oleate, 5.07 (11). This indicates that the excretion of acetone bodies after the caproate and butyrate is an approximately quantitative one, whereas the acetonuria after the administration of the ethyl esters of the fatty acids with 8 or more carbon atoms is greater than that of the acetoacetate controls. This would indicate that more than one acetoacetate residue originates from the oxidation of one molecule of fatty acid having 8 or more carbon atoms.

The relatively low values with ethyl palmitate and ethyl stearate are traceable to the lack of absorption as evidenced by the large excretion of stools in these animals. It has been demonstrated that the administration of the palmitate and stearate in Wesson oil very much increased the absorption of these esters. No acetone bodies were formed after the administration of ethyl palmitate intraperitoneally. The relatively large excretion of acetone bodies following the administration of ethyl oleate indicates that the cleavage of this molecule at the double bond into 2 molecules of nonylic acid cannot be the chief course of oxidation or glycogen should result as the end-product instead of the acetone bodies.

When the ethyl esters of the fatty acids having an odd carbon chain were fed, only insignificant amounts of acetone bodies were excreted in the urine. The average acetone body excretion for the ethyl esters of the fatty acids having odd carbon chains were as follows: propionate, 0.22 (4); valerate, 0.37 (20); heptylate, 0.22

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*The figures in parentheses represent the number of experiments of which this is the average.

(21); undecylate, 0.15 (25); sodium chloride controls, 0.19 (70).

Methyl esters of the fatty acids were found to be somewhat toxic. Experiments are in progress to demonstrate the comparative ketogenic effect of the ethyl esters of palmitic and stearic acids with that of acetoacetate when administered in much smaller doses.

8745 P

Initiation of Lactation in the Hypophysectomized Guinea Pig.

WARREN O. NELSON AND ROBERT GAUNT.

From the Department of Anatomy, Yale University Medical School, and the Department of Biology, Washington Square College, New York University.

The anterior pituitary secretes a hormone concerned in the initiation of lactation. Male or immature female guinea pigs whose mammary glands are developed under the influence of ovarian grafts or oestrin injections lactate when the source of oestrin is removed, but will not do so if the pituitary is simultaneously removed.[1] The following experiments indicate that the adrenal cortex, as well as the pituitary, may be involved in the initiation of lactation in the guinea pig.

Series I. Fourteen guinea pigs were prepared for lactation by prolonged oestrin injections or, in some of the males, by functional ovarian grafts. At the time the ovarian grafts were removed or oestrin injections discontinued hypophysectomy was performed. Injections of a purified preparation of lactogenic hormone* were begun immediately. This treatment in no instance initiated lactation, although continued from 5 to 15 days. In 3 partially hypophysectomized animals lactation was initiated.

Series II. Nine animals similarly prepared for lactation were hypophysectomized and treated with a crude alkaline extract of sheep pituitaries.* This extract contains, in addition to the lactogenic hormone, most if not all of the other known pituitary hormones.

The crude extract, in contrast to the purified lactogenic preparations initiated lactation in the hypophysectomized guinea pigs in every case. Six animals from Group I, failing to lactate on purified lactogenic material, were subsequently treated with crude pituitary extract and lactation was then initiated in every case.

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[1] Nelson, W. O., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 222.

* For the crude pituitary extract, Theelin, and purified lactogenic hormone used here the authors are indebted to Dr. Oliver Kamm of Parke, Davis & Co.

Since the hormonic requirements for the initiation of lactation in the guinea pig after hypophysectomy were not met by the hypophyseal lactogenic hormone alone, a second factor was sought for. A possible explanation was suggested by the observations of lactation deficiencies in adrenalectomized animals,[2, 3] and by the fact that large amounts of the adrenal cortical hormone are necessary for normal lactation in adrenalectomized rats.[3] With this in mind the following experiments were carried out.

Series III. Seven immature males or females were treated for at least 3 weeks with a minimum of 40 R.U. of Theelin* or Amniotin† per day. At the time treatment was stopped hypophysectomy was performed and daily injections of 10 mg. (0.5 rabbit unit) of purified lactogenic hormone plus 2 cc. of adrenal cortical hormone were begun. (The adrenal cortical extracts used here were generously furnished by Dr. W. W. Swingle of Princeton University.) This treatment initiated lactation in every case, and by continued treatment milk could be kept flowing for about 5 days.

Series IV. Three pregnant females were hypophysectomized within 3 days of expected delivery. These animals did not lactate following parturition even when treated with a purified lactogenic hormone. In 2 similar cases, however, in which cortical hormone was added to the lactogenic hormone lactation occurred.

In one case hypophysectomy was performed after lactation had been initiated following parturition. Lactation stopped within 2 days. It was reinitiated after 2 days' treatment with adrenal cortical and purified lactogenic hormones.

Series V. Three animals prepared as in Group III were allowed to go untreated for 4 days after hypophysectomy. After this interval lactation could still be induced by purified lactogenic hormone plus adrenal cortical hormone.

Series VI. Lactation could not be initiated in 3 normal, adult, female guinea pigs treated during and following oestrus with 5 daily injections of 2 cc. of cortical hormone alone. Purified lactogenic hormone is known to induce lactation in such animals.

Lactation once initiated by cortical and lactogenic hormones could

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[2] Brownell, K. A., Lockwood, J. E., and Hartman, F. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 783

[3] Gaunt, R., and Tobin, C. E., *Am. J. Phys.*, 1936, **115**, 588.

† The Amniotin was kindly supplied by Dr. J. A. Morrell of E. R. Squibb & Sons.

not be maintained by cortical hormone alone in the one hypophysectomized animal thus treated from Series III.

Series VII. Lactation could not be maintained (as judged by the death of suckling litters) in 3 hypophysectomized, lactating rats treated with cortical and purified lactogenic hormones. This result was expected in view of the fact that even with crude pituitary extracts we could not maintain lactation in 12 such rats.

Summary. A purified lactogenic hormone, unlike crude pituitary extracts, has failed to initiate lactation in hypophysectomized guinea pigs. Lactogenic hormone plus the adrenal cortical hormone (Swingle-Pfiffner extract) has initiated lactation in such animals in every case.‡

8746 C

Production of Shwartzman Reaction in Rabbits with Purified Fraction of *B. coli* Filtrate.

H. B. ANDERVONT AND M. J. SHEAR.

From the Office of Cancer Investigations, U. S. Public Health Service, Harvard Medical School, Boston.

Previous publications[1, 2] from this laboratory described a method for the separation and concentration of the factor in *B. coli* filtrates which produces hemorrhage in mouse tumors. Since *B. coli* filtrates are known to produce the Shwartzman reaction in rabbits, it was of interest to ascertain whether the purest fraction of the tumor-affecting agent obtained thus far[2] was also capable of eliciting hemorrhage in prepared sites in the skin of rabbits. It was also desirable to make quantitative comparisons between the original filtrate and the purified fraction as to their ability to produce hemorrhage in tumors of mice as well as the Shwartzman reaction in the rabbit.

Equivalent tumor-affecting doses were determined by performing a preliminary titration in mice bearing cutaneous tumors, according to a technic described elsewhere.[3] Strain M mice bearing 6-day-old cutaneous growths of sarcoma 37 were given intra-abdominal injections of diluted filtrate or dilutions of the purified fraction. All dilutions were made with 0.9% NaCl solution. It was found that 0.2 cc. of a 1:50 dilution of the original filtrate produced hemorrhage in tumors, but 0.2 cc. of a 1:100 dilution failed to do so. The purified fraction was active down to 0.2 cc. of a 1:1500 dilution of a solution which contained 2 mg. of the purified fraction per cc. Thus the minimal tumor-affecting dose of this purified fraction was 0.26γ.

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‡ Judging by gross observation at autopsy, anterior lobe removal was complete in all of these cases. To verify this, however, sections of the sella turcica contents have been made in most cases, although not yet complete in a few cases. We have omitted in this account those animals, a considerable number, that failed to survive the operation long enough to allow for the necessary observations.

[1] Shear, M. J., and Andervont, H. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 323.

[2] Shear, M. J., *ibid.*, 1936, **34**, 325.

Comparable doses of the two materials were then injected into rabbits according to Shwartzman's technic. The endermal injections consisted of 0.2 cc. doses. Each of 4 rabbits received preparatory injections of 3 dilutions of the original filtrate and of 3 dilutions of the purified fraction, as shown in Table I. Twenty-four hours later 2 of the rabbits each received 0.5 cc. of undiluted filtrate intravenously while the other 2 received 0.5 cc. of a 1:10 dilution of the purified fraction. The results are shown in Table I.

It is seen that both the filtrate and the purified fraction elicited

TABLE I.

Response of Cutaneously Prepared Rabbits to Intravenous Injection of *B. coli* Filtrate and of a Purified Fraction Obtained from the Filtrate.

		Preparatory endermal injection (0.2 cc.) in all 4 rabbits					
		Original filtrate (undil.)	Original filtrate (1:10)	Original filtrate (1:50)	Purified fraction, 0.04 mg.	Purified fraction, 0.002 mg.	Purified fraction, 0.0005 mg.
Rabbit No.	Intravenous injection	Results					
467	Purified fraction, 0.1 mg.	+++	+++	+++	++++	+++	++
468	Purified fraction, 0.1 mg.	++	+	—	+++	++	+
469	Original filtrate, 0.5 cc. (undiluted)	++	—	—	+++	—	—
470	Original filtrate, 0.5 cc. (undiluted)	+++	—	—	++++	—	—

Note: In the above table + denotes a positive Shwartzman reaction and — a negative Shwartzman reaction. The number of + signs are used to denote the relative size of the areas covered by the reaction.

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[3] Andervont, H. B., *Am. J. Cancer*, 1936, **27**, 77.

the Shwartzman reaction in rabbits. However, the purified fraction appears to be more potent than the filtrate in producing the reaction, since intravenous injection of a 1:10 dilution of the stock solution of the purified fraction produced the reaction at practically all sites of endermal injection of both filtrate and of purified fraction while intravenous injection of the undiluted filtrate elicited the reaction only in those sites that had received the highest concentration of filtrate or of purified fraction.

It is of interest to note that, so far as the purified fraction is concerned, the greater the amount of tumor-affecting agent employed in the preparatory injections the more pronounced is the Shwartzman reaction. It may also be pointed out that, in this experiment, a positive Shwartzman reaction was obtained with an amount of purified fraction (0.0005 mg.) that is close to the minimal amount (0.0003 mg.) effective in eliciting hemorrhage in mouse-tumors.

8747 P

Chemical Composition of *Bacterium tumefaciens.*

ERWIN CHARGAFF AND MICHAEL LEVINE.

From the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University, and Montefiore Hospital, New York.

Bacterium tumefaciens is the etiological agent of plant tumors, known as crown-gall disease.[1] The importance of investigating the chemical composition of this organism and the pathological effects of its various constituents is apparent. Although the etiology of plant tumors probably does not parallel that of animal cancer, the fact that a chemical stimulant of cellular proliferation apparently is synthesized by this microörganism is reminiscent of certain results obtained with chemically well-defined carcinogenic agents. Doubtless, a deeper knowledge of the mechanism underlying the development of plant cancer would also be of value for a better understanding of malignant growth in general.

A virulent strain of *B. tumefaciens* (strain 6NIS[6]) was cultivated on sterile bean-broth in the dark at room temperature for 14 days. The washed bacteria were suspended in a mixture of equal amounts of alcohol-ether which extracted the fat and phosphatide. The defatted organisms then were extracted with chloroform, in order to remove the wax fraction which, however, in this bacterium is very small and was not further examined. The separation and purification of the lipids were carried out according to the method of Anderson.[2] The distribution of lipids in 3 batches of bacteria of the same strain grown at different times is given in Table I. It will be seen that the results are remarkably uniform. The dry weight of the bacteria examined, in each case, was between 2 and 3 gm.

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[1] For discussion and literature compare M. Levine, *Am. J. Cancer*, 1931, **15**, 1410.

TABLE I.
Lipids of *Bact. tumefaciens* (in % of total dry bacteria).

Fraction	Experiments I	II	III
Fat	5.7	5.8	5.6
Phosphatide	1.6	2.0	1.8
Wax	0.8	—*	—*
Bacterial residue	91.9	92.2	92.6

*Not isolated.

The *acetone-soluble fat* is a yellow viscous oil. Its constants, determined according to the micro-method described by one of us[3] were: iodine No. 95.0, saponification No. 201.2, acid No. 53.2, ester No. 148.0. On saponification (carried out in N_2) the following components were obtained from 174 mg. of fat: 15.6 mg. of unsaponifiable matter (9% of the fat) and 142.9 mg. of fatty acids (82.1% of the fat). The unsaponifiable fraction showed no selective absorption in the ultraviolet region in alcohol-chloroform (2:1) solution; there was only an end-absorption around 2350 Å. About 10% of the unsaponifiable fraction consisted of a sterol mixture which melted unsharply around 80°, was precipitated by digitonin and gave a positive Liebermann-Burchard reaction and a negative Rosenheim reaction. This sterol may have been carried over from the medium. The fatty acids (142.9 mg.) were separated into the solid and liquid acids according to the Twitchell procedure. The solid acids weighed 30.0 mg. and had the iodine value 43.3; the liquid acids weighed 106.4 mg. and had the iodine value of 51.6. The fat apparently contains unsaturated fatty acids of high molecular weight, as is also the case with the fat of *C. diphtheriæ*.[4]

The *phosphatide* was purified by repeated precipitation from its ethereal solution by means of acetone. It formed in the cold a somewhat sticky yellow powder which on slow heating started to soften at 67° and melted completely at 125°. It contained N 2.2%, P 3.4%.

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[2] Anderson, R. J., *J. Biol. Chem.*, 1927, **74**, 525.

[3] Chargaff, E., *Z. physiol. Chem.*, 1931, **199**, 221.

[4] Chargaff, E., *Z. physiol. Chem.*, 1933, **218**, 223.

From the defatted bacteria a *polysaccharide* was obtained which amounted to about 3.5% of the material. This substance contained 65.3% of reducing sugars (calculated as glucose).

A detailed report on the chemical composition of the fractions mentioned and on their biological behavior will be presented at a later date.

The various substances isolated are being tested, at present, as to their effect on the Paris daisy, castor bean, geranium, Bryophyllum and two species of tobacco. Preliminary tests made on the Paris daisy, geranium and Ricinus indicate that the phosphatide acts as a growth-stimulant, whereas the fat is much less active.

8748 C

Some Physical and Chemical Properties of Desiccated Stomach.

JEAN KYER, FREDERICK P. BROOKS AND RAPHAEL ISAACS.
From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan, Ann Arbor.

Since the introduction of the use of desiccated hog stomach in the treatment of pernicious anemia by Sturgis and Isaacs,[1] Sharp,[2] Conner,[3] and Wilkinson,[4] efforts have been made to fractionate the material and determine the nature of the active principle. In the following experiments whole hog stomach was finely ground, dried at a temperature below 30°C., and the fat extracted with petroleum benzine. With this method 100 gm. of fresh stomach yield 14-15 gm. of dry powder. This material is stable, retaining its anti-anemic potency for months. It is non-hygroscopic but on the addition of water swells and forms a milky suspension.

This material was used as a starting point for further manipulations. In each case the substance was given to one or more suitable patients with pernicious anemia having the following qualifications: a macrocytic anemia with a red blood cell count of less than 2.5 millions per cubic millimeter; no free hydrochloric acid in the stomach after histamine injection intramuscularly; no previous antianemic medication for at least one month; hospitalization with close observation during treatment; no gross infection; no evidence of inability to absorb material given by mouth (normal gastric emptying time); corroborative clinical data of pernicious anemia.

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[1] Sturgis, C. C., and Isaacs, R., *J. A. M. A.*, 1929, **93**, 747.

[2] Sharp, E. A., *J. A. M. A.*, 1929, **93**, 749.

[3] Conner, H. M., *Proc. Staff Meet. Mayo Clinic*, 1929, **4**, 319.

[4] Wilkinson, J. F., *Brit. Med. J.*, 1930, **1**, 236.

Studies of the properties of desiccated stomach show that it contains pepsinogen, that the peptic activity (Mett's test) is absent in fresh aqueous filtered solutions, and that the aqueous filtrate of 5 gm. of desiccated stomach in 50 cc. of distilled water develops an activity equivalent to 0.5% pepsin when acidified with HCl.

A 1:100 aqueous extract of desiccated stomach dried in open pans in the air current from an electric fan (5.13 gm. = 40 gm. of desiccated stomach tissue) gives the calculated hematopoietic response when fed to a patient with pernicious anemia. The dried aqueous extract retains its peptic activity but is inactivated after treatment with acetone. Prolonged contact with acetone inactivates both the pepsin and the hematopoietic property, the degree of inactivation being proportional to the time of contact. Ultrafiltration (pyroxylin-Berkefeld filter) also removes the peptic activity and the hematopoietic activity.

A watery suspension acidified to pH 4.5 with HCl gives a clear yellow filtrate which is as active in the treatment of pernicious anemia as a dried aqueous extract. This acid extract also has peptic activity. Heating an aqueous suspension or an acid extract of stomach to 60°-70°C. for half an hour inactivates it for both its peptic activity and its ability to induce a hematopoietic remission in a patient with pernicious anemia.

Although the peptic activity and the hematopoietic activity parallel one another, specimens of desiccated stomach inactivated by heat do not regenerate their hematopoietic activity after incubation with commercial pepsin.

In determining the effect of alkali upon the active principle, 500 gm. of desiccated stomach were suspended in 5 liters of water, made alkaline with NaOH to pH 9.0, and stirred for an hour. The mixture was filtered and the filtrate, after neutralization to pH 7.0, was fed daily in 200 cc. amounts to a patient with a red blood cell count of 1.38 millions per cubic millimeter producing a maximum reticulocyte response of 25% in 7 days.

It is known that fresh human gastric juice alone does not produce a response when fed to a patient with pernicious anemia,[5, 6] so

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[5] Castle, W. B., and Townsend, W. C., *Am. J. Med. Sci.*, 1929, **178**, 764.

[6] Helmer, O. M., Fouts, P. J., and Zerfas, L. G., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 775.

desiccated stomach and its potent extracts must supply another factor in addition to the enzyme-like substance contained in gastric juice. The following experiment was planned to determine the nature of the other factor which with the enzyme-like substance is the active principle of the stomach tissue.

When an acid extract (pH 4.5) of 400 gm. of desiccated stomach was boiled for 5 minutes the clear yellow solution became cloudy but did not coagulate, so it was concentrated to a small volume, made 60% alcoholic, and filtered. The filtrate was concentrated under reduced pressure to remove the alcohol and 40 cc. (representing 56 gm. of desiccated stomach) were fed daily to a pernicious anemia patient, with no hematopoietic response. After 7 days 40 cc. of the same material were incubated for 3 hours with 200 cc. of human gastric juice and fed to the same patient at 8 o'clock every morning, breakfast being omitted. The red blood cell count was 1.43 millions per cubic millimeter and the number of reticulocytes was 15.3% in 9 days. It is thus evident that a second substance necessary for the development of the anti-anemic principle is present in stomach tissue. This substance is heat-stable and probably interacts with the heat-labile factor in a manner similar to the behavior of the "extrinsic" (heat-stable) and "intrinsic" (heat-labile) factors postulated by Castle and Strauss.[7]

The next series of experiments was designed to determine the effect of incubation on stomach extracts and to prepare products suitable for intramuscular injection.

An acid extract of desiccated stomach was made 70% alcoholic, evaporated to a small volume *in vacuo,* and sterilized by filtration through a Berkefeld filter. Such a preparation, without the sterilization, had been inactive when administered orally and it was desired to determine its activity when injected. The equivalent of 120 gm. of desiccated stomach was given intramuscularly over a period of 5 days to a patient with no hematopoietic response.

An acid extract was incubated alone for 8 hours, made 70% alcoholic, and after the alcohol was removed the solution was boiled and evaporated to a small volume. The equivalent of 175 gm. of desiccated stomach was given intramuscularly over a period of 7 days to a patient with no hematopoietic response.

At this time we observed that Wilkinson and Klein[8] had prepared an active intramuscular product, which was soluble in 70% alcohol and which would resist a temperature of 60°-65°C. for 30 minutes, by incubation of a stomach product with beef muscle. It appeared then that our preparation might also resist a temperature of 60°-65°C., although not a boiling temperature.

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[7] Castle, W. B., and Strauss, M., *New Eng. J. Med.*, 1932, **207**, 55.

[8] Wilkinson, J. F., and Klein, L., *Lancet*, 1933, **2**, 629.

Consequently, an acid extract was incubated 8 hours, heated at 60°-65°C. for 30 minutes, made 70% alcoholic, and worked up into a product suitable for intramuscular injection. When the equivalent of 75 gm. was administered to a patient over a period of 3 days the reticulocytes rose from 4.5% to 15.9% in 7 days (R.B.C. = 1,400,000 per cu. mm.).

From these experiments it is evident that the incubation of an acid solution of stomach tissue alone produces a substance which is not identical with the active principle of liver (because it is destroyed by boiling) but which is possibly some intermediate product between the original principle in stomach and the heat-stable principle found in liver.

Summary. A water extract of desiccated hog stomach acidified to pH 4.5 is active in the treatment of pernicious anemia. The hematopoietically active substance does not pass through the ultrafilter and is stable toward alkali. Stomach extracts inactivated by heat do not regenerate their hematopoietic activity by treatment with pepsin. An acid extract contains 2 factors, one heat-stable and the other heat-labile, both of which are necessary for the production of a reticulocyte response in a patient with pernicious anemia. An acid extract incubated for 8 hours produces a hematopoietically active substance which is soluble in 70% alcohol and which resists a temperature of 60°-65°C. for 30 minutes, but not a boiling temperature. Incubation in this manner produces a substance which is not so heat-stable as the anti-anemic principle in liver but which may be an intermediate product more heat-stable than the original stomach fractions.

8749 P

Response of the Iris to Pitressin and Pitocin.

KENNETH C. SWAN AND HAROLD B. MYERS.

From the Department of Pharmacology, University of Oregon Medical School, Portland.

Posterior pituitary extract (pituitrin) has been reported[1] to cause a dilatation of the pupil when dropped into the conjunctival sac of the normal eye of the rabbit, averaging 0.65 mm. (minimum: 0.25; maximum: 1.5) in 31 of 32 experiments. In one of 32 experiments the pupil was found to contract and stay contracted following the use of pituitrin.

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[1] Pollock, W. B., *Brit. J. Ophthalmol.*, 1920, **4**, 106.

In 8 experiments we have found the posterior pituitary extract prepared by 2 manufacturers (4 experiments with each) to cause a constriction of the pupil of the normal eye of the rabbit amounting to 1.0 mm. in each of the 8 experiments.

The active constituents of the posterior pituitary have been separated into 2 fractions, one possessing vasopressor, the other oxytoxic actions.[2] These fractions are commonly available under the names "pitressin" and "pitocin", respectively. Pitressin in the normal rabbit eye caused a constriction in each of 7 experiments, averaging 2.2 mm. (minimum: 1.5; maximum: 4.0). Pitressin placed in the conjunctival sac following the use of 1% atropin until the light reflex was abolished, caused a constriction of the pupil in each of 9 experiments, averaging 4.9 mm. (minimum: 4.0; maximum: 6.3).

Pitressin placed in the conjunctival sac following pronounced dilatation of the pupil from 4% cocaine caused constriction of 6.5 mm. in each of 2 experiments. Pitressin following ephedrine 3% solution, resulting in dilatation of the pupil averaging 2.0 mm. increased diameter, failed to influence the size of the pupil in each of 4 experiments. Pitressin following epinephrine 1:1,000 solution, resulting in 0.5 mm. dilatation of the pupil, caused constriction of 1.0 and 1.5 mm. in 2 experiments.

Pitocin in the normal rabbit eye caused no change in the size of the pupil in each of 7 experiments. Pitocin in the eye with pupil inactive to light following atropine failed to alter the size of the pupil in each of 3 experiments.

Pitocin (10 unit strength) when mixed with pitressin (10 unit strength) did not appear to influence the degree or duration of pitressin constriction of the normal rabbit pupil (average: 2.6 mm.; minimum: 1 mm.; maximum: 3.1 mm.) in 4 experiments. Pitocin in 3 experiments did not appear to alter the action of pitressin in constricting the pupil dilated with atropine.

All observations were made with uniform artificial light.

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[2] Kamm, O., Aldrich, I. W., Grote, I. W., Rowe, L. W., and Bugbee, E. P., *J. Am. Chem. Soc.*, 1928, **50**, 573

8750 C

Fasting Ketosis in the Pregnant Rat as Influenced by Adrenalectomy.

Eaton M. MacKay and Richard H. Barnes.
From the Scripps Metabolic Clinic, La Jolla, California.

It is generally believed that in the human being ketonuria is more easily produced in pregnancy and it has been shown[1, 2] that ketosis is provoked more rapidly and easily by carbohydrate starvation in this condition. However, Harding and Allin[3] believe that it develops only in accordance with Shaffer's theory. Burn and Ling[4] found that rats on a butter fat diet have a sharp rise in ketone body excretion during the several days of pregnancy before parturition after which it drops to a normal value. Butts, Cutler and Deuel[5] found an excessively high ketone body excretion for one day in a fasting pregnant rat. In Table I we have demonstrated the

TABLE I.

		Day				Day litter born
No.	Body wt.	1	2	3	4	
	gm.	ketone bodies—mg.				
Group A—Pregnant						
1	215	1.7	97.6		149.0	6
2	242	35.6	167.0	127.0	90.5	5
3	230	0.0	176.0	6.0		6
4	205	0.0	99.4	37.7		4
5	216	3.5	93.9			2
Group B—Non-pregnant						
6	206	1.7	1.2	0.6	0.9	
7	204	1.2	1.4	1.0	2.3	
8	199	1.2	1.1	0.4	1.7	
Group A2—Non-pregnant (same rats as Group A)						
1	180	1.4	1.0	1.7	1.0	
2	210	2.1	1.4	2.8	1.5	
3	168	1.0	1.2	2.0	0.9	
4	168	1.0	1.2	2.2	1.3	
5	158	1.0	0.6	0.6	1.3	
Group C—Pregnant and Adrenalectomized						
9	255	0.5	0.8	4.1	0.8	2
10	240	0.9	4.3	1.8	1.1	3
11	228	0.7	2.1	4.3	0.7	5
12	203	0.2	2.4	0.3	0.6	6

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[1] Bokelmann, O., and Bock, A., *Arch. Gynakol.*, 1926-27, **129**, 542.

[2] Bokelmann, O., and Bock, A., *Z. Geburtsh. u. Gynakol.*, 1927-28, **92**, 184.

[3] Harding, V. J., and Allin, K. D., *J. Biol. Chem.*, 1926, **69**, 133.

[4] Burn, J. H., and Ling, H. W., *Quart. J. Pharm. and Pharmacol.*, 1933, **2**, 1.

[5] Butts, J. S., Cutler, C. H., and Deuel, H. J., Jr., *J. Biol. Chem.*, 1934, **105**, 45.

large fasting ketosis during the few days before parturition (Group A) in comparison with non-pregnant, adult, female rats fasted at the same time (Group B) and the same rats that were pregnant, when fasted 2 weeks after parturition (Group A2). Their young had not been allowed to nurse but were taken away at birth. The high fasting ketosis of pregnancy is quite obvious (Group A).

The possibility exists that the increased degree of ketosis during pregnancy results from excessive production of the so-called ketogenic principle of the anterior pituitary and we know that in other respects the anterior pituitary is very active at this time. In another note[6] we have shown that this ketogenic principle is not active in the absence of the adrenal glands, the necessary part of the latter apparently being the cortex. We have examined the effect of adrenalectomy upon the fasting ketosis in pregnancy (Group C) and find that it does not occur if the adrenals have been removed.

We have suggested that the marked fasting ketosis of pregnancy may be due to an accentuation of anterior pituitary activity. The failure of pregnant rats to develop a significant ketosis in the absence of the adrenal glands lends indirect support to this view.[6] A finding which may have a bearing on this point is the observation of Butts *et al.*[5] that the pituitary-like gonadotropic factor from the urine of pregnant women ("Antuitrin S" of Parke, Davis & Co.) produces no ketosis and we have confirmed their experiments. The combined gonadotropic and ketogenic activity of anterior pituitary extracts means little because of our inability to definitely purify such preparations.

Summary and Conclusions. Fasting pregnant rats develop a ketosis of large magnitude when compared with non-pregnant controls. Removal of the adrenal glands abolishes this fasting ketosis in pregnant animals.

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[6] MacKay, E. M., and Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1936,

8751 P

Neoplastic Hyperplasia of Gastric Mucosa in Rats.*

GEORGE R. SHARPLESS. (Introduced by F. W. Hartman.)

From the Department of Laboratories, Henry Ford Hospital, Detroit.

This is a report of the production and prevention of morphological changes in the fore-stomach of rats by means of the diet.

Pappenheimer and Larimore[1] described lesions in the fore-stomachs of rats which appeared as round or oval discs with a small central ulcerated depression. The cause of the lesion was not found, but excess base or acid or Vitamin A deficiency were found not to be the etiological agents. Findlay[2] described changes in the fore-stomach associated with Vitamin B deficiency. Fujimaki has published a number of reports on the production of similar morphological changes in the fore-stomachs of rats by feeding different purified diets. His first hypothesis[3, 4] was that the condition was caused by Vitamin A deficiency, but later[5, 6] found that butterfat and/or cod liver oil caused a more marked change. The conclusion was drawn[6] that any of a number of fatty acids might play a partial etiological rôle.

The basal diet used to produce the lesion described here is as follows: Casein (vitamin free) 4.0%; dried brewer's yeast 5.0%; salts McCollum No. 185, 4.0%; butterfat, 5.0%; carbohydrate (starch, sugar, or dextrin), 82.0%; viosterol (15 drops per kilo of food).

Rats 28 to 35 days old, weighing 60 to 110 gm. were used. The lesion develops within 3 months, and all of the animals on the above diet which have been examined to date (40) have been affected.

The macroscopic appearance of the lesion varies from a few small elevated round or oval discs to long ridges of thickened epithelium with small papillomatous projections. There is a depression in the center of ridges and discs which may show ulceration. The normal fore-stomach is thin and elastic, but in animals with a severe lesion the epithelium is so greatly thickened that its elasticity has been lost.

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* Presented before American Society of Experimental Pathology, Washington, D. C., March 26, 1936.

[1] Pappenheimer, Alwin M., and Larimore, Louise D., *J. Exp. Med.*, 1924, **40**, 719.

[2] Findlay, G. M., *J. Path. and Bact.*, 1928, **31**, 353.

[3] Fujimaki, Yoshitomo, *J. Cancer Res.*, 1926, **10**, 469.

[4] Fujimaki, Yoshitomo, *Gann*, 1927, **21**, 8.

[5] Fujimaki, Yoshitomo, *Trans. Jap. Path. Soc.*, 1928, **18**, 548.

[6] Fujimaki, Yoshitomo, *Trans. Jap. Path. Soc.*, 1931, **21**, 708.

Older lesions may show from 2 to 8 epithelial cysts which vary in size up to 6 mm. in diameter. The most frequent site of occurrence is near the ridge separating the fore-stomach from the glandular portion, but it may occur in any one or all parts of the fore-stomach.

Microscopically, the lesion is characterized by a greatly thickened squamous epithelium which may show papilloma formation with keratinization at the surface. The papillae are markedly hypertrophied and may show active proliferation, numerous mitotic figures, and epithelial cells with hyperchromatic nuclei. In some cases the epithelial papillae show active proliferation with invasion of the submucosa. Ulceration of the epithelium may occur and epithelial cysts walled off by squamous epithelium and filled with keratin may be present.

Twenty-five rats fed the basal diet with 12.0% casein instead of 4.0% had normal stomachs.

Six rats, from 2 litters, fed the basal diet with 0.2% cystine and 6 fed the basal diet with 0.05% methionine did not develop the lesion, whereas littermate controls fed the basal diet alone invariably showed the characteristic changes. Cysteine hydrochloride and glutathione have also been tried but have not prevented the development of the lesion.

The rats nearly always have some hair in their stomach contents, which may act as an irritant and thus be a factor contributing to the production of the lesion. However, there is as much hair in the stomachs of those that do not develop the lesion as in those that do; thus it cannot be the primary etiological factor.

These rats are not in a chronic state of Vitamin A deficiency; their livers show abundant Vitamin A by the antimony-trichloride test, and supplementing the diet with carotine has not prevented the condition. Pappenheimer and Fujimaki have also found that Vitamin A deficiency is not a factor in the development of the lesion.

Metastases have not been observed in any of these rats.

These observations indicate that neoplastic hyperplasia of the gastric mucosa of rats may be produced by feeding a diet low in casein and prevented by supplementing the diet with cystine or methionine. These changes are apparently unrelated to Vitamin A deficiency.

8752 C

Influence of Climate on Susceptibility of Monkeys to Intranasal Infection with Poliomyelitis Virus.

Albert B. Sabin. (Introduced by Peter K. Olitsky.)

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

Although sporadic cases of poliomyelitis occur throughout the year, the incidence of the disease rises sharply during the late summer (and early autumn months) and, as a rule, decreases with the onset of cold weather. The cause of this seasonal incidence is as yet obscure. The virus is supposedly transmitted from man to man by droplet infection without the intervention of an intermediary host or reservoir upon which changes in climate could exert an effect. That droplet infection is not in itself the factor is clear from the well-known fact that other diseases believed to be transmitted in the same manner have their own peaks of seasonal incidence, many of which vary and happen to be different from that of poliomyelitis.[1] Since the virus of poliomyelitis is less resistant to higher than to lower temperatures, it does not seem probable that the greater occurrence of the disease during the hot months is attributable to an effect of climate on the virus itself. Hence it appears that the host, more than any other factor, should be considered as the agent influenced by climate, although the nature of this possible influence remains unknown. Presumably the climatic effect may be either a local one, *i. e.,* the nasal secretions may perhaps be so changed as to prevent the virus from gaining access to the first cells (olfactory) which it apparently must infect in order to spread further to the central nervous system, or the change may be elsewhere along the course of the virus, which would impede its progress and thus prevent the disease from becoming clinically manifest.

The question which this investigation proposed to examine was whether or not cold weather or changes from warm to cold could in themselves so influence the nasal membranes or secretions as to render infection with the virus of poliomyelitis more difficult. Six *Macacus rhesus* monkeys were kept outdoors 8 to 9 hours each day during late February and March, when it snowed on 2 days and the temperature varied from 28° to 50°F. During the remainder of the day they were kept indoors in a room in which the temperature is automatically regulated at 72° to 76°F. They were purposely allowed to spend part of the time indoors in order to approximate more closely living conditions of man during cold weather. The monkeys were outside every day for a week before they were given poliomyelitis virus intranasally. One cc. of a 10% suspension of pooled poliomyelitic monkey cords was instilled in each nostril on two occasions 48 hours apart. Subsequent to this treatment they remained outside during the day until the end of the experiment. Seven monkeys which were kept indoors in the same room during the same period as the others were similarly inoculated and the course of the disease in the two sets of animals observed (Table I).

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[1] Poliomyelitis, International Committee for the Study of Infantile Paralysis, Williams and Wilkins Co., Baltimore, 1932, p. 335.

TABLE I.

Group	Monkey No.	Result
Outdoors 8-9 hours each day for 1 week prior to inoculation and for duration of experiment. Temperature during period, 28° to 50° F.; average, 38.9° F.	7-52	Fever 4, Par. 8, Prostr. 9*
	7-53	” 4, ” 11, ” 12
	7-54	” 4, ” 8, ” 11
	7-55	” 4, ” 7, ” 7
	7-56	” 4, ” 8, ” 9
	7-57	” 4, ” 8, ” 8
Indoors for 1 week prior to and throughout course of experiment. Average temperature, 74° F.	7-58	” ?, ” 9, D. 10
	7-59	” 4, ” 7, Prostr. 8
	7-60	” ?, ” 12, ” 14
	7-61	Dead 14th day of Tbc. No clinical or histological signs of poliomyelitis
	7-62	Fever 4, Par. 8, Prostr. 9
	7-63	” 5, ” 8, ” 9
	7-05	” 6, ” 9, ” 10

*Fever 4, Par. 8, Prostr. 9 = Fever 4th day, paralysis 8th day, complete paralysis with subnormal temperature 9th day after first nasal instillation of virus.

It is obvious that under the conditions of the experiment the changes in climate to which the monkeys were submitted failed to diminish the incidence of experimental poliomyelitis among them. All six developed the disease and exhibited signs somewhat more rapidly than their mates which were kept under fairly constant temperature indoors. While these results do not exclude the possibility that cool or cold weather may affect conditions in the nose of man in a different manner, it seems more probable that the influence of climate is exerted on other factors in the host.

To elucidate this problem further, other studies are being considered which aim to determine (by means of antibody tests on children before and after the months of highest incidence) whether the total number of cases of poliomyelitis, both recognized and inapparent, is increased during the late summer and early autumn, or whether the climatic influence is merely to augment the number of clinically apparent cases without appreciably changing the total number infected.

8753 C

Concentration of a Hyperglycemic Factor from Urine.

BENJAMIN HARROW, ABRAHAM MAZUR, I. M. CHAMELIN AND ALEX LESUK.

From the Chemical Laboratory, College of the City of New York.

We have previously reported the preparation of a crude urine (male) extract which, when injected into rabbits, causes a hyperglycemia, an increase of acetone bodies and lactic acid in the blood, and a decrease of CO_2-combining power.[1] We now wish to report appreciable progress in the purification of the material responsible for the hyperglycemic effect. We are not as yet prepared to state whether our purified material will respond to the other tests of our earlier (crude) product. The crude substance, in the dry state, can be kept for a considerable time without any marked deterioration. The active material is dialyzable and can be further purified by removing the inorganic sulfates. From approximately 250 liters of urine (male), 52 mg. of a highly active, rather unstable organic substance have been isolated. The amount so far obtained was sufficient for physiological tests (confined to blood sugar determinations) but not for chemical identification.

The flow-sheet on the following page illustrates the method of preparing this highly active material.

Since various modifications in the procedures previously described have been adopted, we shall give specific details:

The urine used is obtained from men of 17-22 years of age. It is preserved with thymol and kept in the ice-box. Urine older than 3 days is discarded.

The urine is acidified to methyl red (pH 5) with glacial acetic acid. A warm solution of benzoic acid in alcohol (30 gm. benzoic acid in 60 cc. 95% ethyl alcohol per liter of urine) is added, drop

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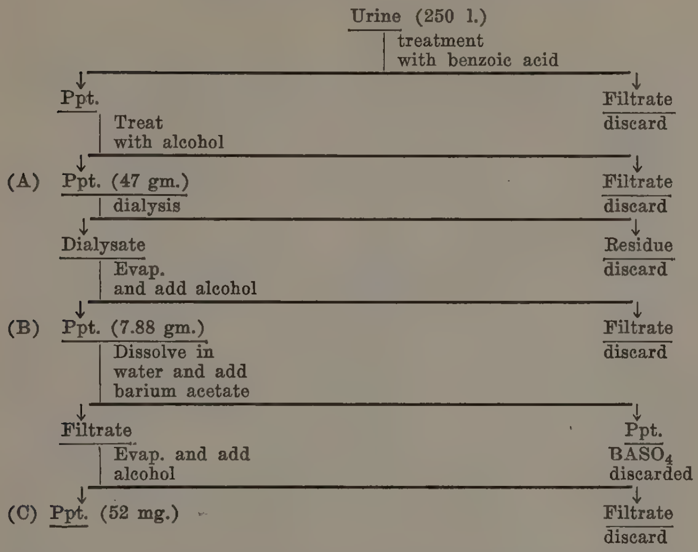
[1] Harrow, B., *Science*, 1934, **79**, 272; Harrow, B., Naiman, B., Chamelin, I. M., and Mazur, A., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 940: Harrow, B., Chamelin, I. M., and Mazur, A., *Am. J. Physiol.*, 1934, **109**, 436.



by drop, with vigorous (mechanical) stirring. The precipitate is filtered, sucked dry on a Buchner funnel, extracted with alcohol (60 cc. per liter of urine) and centrifuged. (The discarded alcohol-soluble fraction contains benzoic acid, male hormone, etc.) The precipitate is further washed with small portions of alcohol (a total of 30 cc. per liter of urine), twice with ether, centrifuged and dried in a vacuum desiccator. This is fraction (A). This fraction gives positive biuret, Hopkins-Cole and Millons, questionable ninhydrin and Molisch and negative Benedict's. It gives a weak labile sulfur test and a precipitate with phosphotungstic acid. Its activity is equal to 1.1 units per gm. (See Table I.)

We define our unit of activity as equal to that weight (in gm.) of material which, when injected subcutaneously into a 2 kg. female, fed rabbit, produces a 100% increase in blood sugar within the first 3 hours. An average of 3 determinations on different rabbits is taken.

For further purification, (A) is triturated with water (7.5 cc. per gm.) containing a little thymol, and the suspension is dialysed in a cellulose (Visking) membrane against water (15 cc. per gm.). The dialysis is continued for 3 days, the outside solution being replaced with fresh water at the end of each 24-hour period. This dialysis is carried out in the ice-box at 5-10°C.

The combined dialysates are evaporated *in vacuo* to a small volume

TABLE I.

Fraction	Test sample mg.	Glucose	% increase Aver.	Activity per gm., units	Total activity, units
A					
Alcohol-insoluble crude	500	58.7	56.4	1.1	51.7
urine extract, 47 gm.	"	60.1			
from 250 l. urine	"	50.4			
B					
Alcohol-insoluble dialysate	50	23.0	23.5	4.7	38.6
7.88 gm. solids		24.0			
	"	29.7	28.8	5.8	
		27.8			
	"	21.9	21.0	4.2	
		20.0			
C					
Alcohol-insoluble; free	2.6	22.3	23.0	88	4.3
from inorganic salts,		23.7			
52 mg. solids	7.5	56.7	55.2	74	
		53.7			
	13.0	123.2	114.7	88	
		106.2			

(keeping the temperature below 37°C.) and alcohol is added to make a 95% solution; it is kept in the ice-box overnight. The material is centrifuged, washed with alcohol and ether and dried in a vacuum desiccator. This is fraction (B). It gives negative protein tests but contains a large percentage of potassium sulfate. Its activity is equal to 4.9 units per gm. (See Table I.)

The next step in the purification is to remove the sulfates. Most of (B) is soluble in water. The $SO_4^{=}$ in the solution is quantitatively removed with barium acetate, the filtrate, free from $BaSO_4$, is evaporated *in vacuo* to a small volume, and alcohol is added to make a 95% solution; it is kept in the ice-box overnight. The precipitate which forms is centrifuged, washed with alcohol to remove the last traces of potassium acetate, then with ether and dried in a vacuum desiccator. This is fraction (C). Its activity is equal to 83 units per gm. (See Table I.) It appears to be entirely organic in nature and somewhat unstable on drying, turning brown and gummy.

Table I gives a summary of the results.

Summary. 1. We have concentrated our active material from an activity of 1.1 units per gm. to 83 units per gm. 2. Approximately 250 liters of male urine yields 52 mg. of this highly active, somewhat unstable substance.

8754 C

Comparative Studies of Gonadotropic Hormones.* 4. Ovaries and Hypophyses of Rats in Chronic Experiments.

C. F. Fluhmann.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

In a previous report[1] some significant differences in 3-week experiments were noted between rats given a gonadotropic preparation made from human pregnancy blood and rats injected with an extract of sheep anterior pituitary material. The present study deals with the changes resulting in the ovaries and hypophysis from the use of such hormones for periods varying from 2 months to one year.

Both preparations were made in the same way, by extracting with a sodium acetate-acetic acid buffer, pH 4.2 to 4.5 and reprecipitating with ethyl alcohol, according to the method previously described.[2] In all cases the experiments were begun when the rats were 20 to 23 days of age. A single daily injection of 0.25 cc. of each extract was used, and the controls consisted either of small numbers of litter-mates or larger groups of rats of the same age.

Sheep Anterior Pituitary Extract. When given to immature rats once daily for 5 days, the sheep anterior pituitary extracts produced an average increase of ovarian weight varying from 70 to 267%. In 63 days, the average increase in the ovarian weight of 6 rats was 153% (body weight 135 gm., ovaries 0.114 gm.), and after 94 days the ovaries of 3 animals had an average increase in weight of only 57%, while the anterior pituitary gland gave an increase of 44% (body weight 174 gm., ovaries 0.107 gm., hypophysis 0.013 gm.). The continued daily administration of the extract resulted in a further decrease in the size of the ovaries, while the hypophysis remained larger than normal. After 150 days the ovaries of 5 rats gave a 47% diminution in weight as compared to the controls, while the hypophysis showed a 27% increase (body weight 190 gm., ovaries 0.031 gm., hypophysis 0.014 gm.). Five rats injected for 179 days gave an average diminution in ovarian

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* Supported in part by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine. The sheep anterior pituitary glands were supplied by Messrs. Parke, Davis & Company, through the courtesy of Dr. E. A. Sharp.

[1] Fluhmann, C. F., *Am. J. Physiol.*, 1933, **106**, 238.

[2] Fluhmann, C. F., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 1193.

weight of 37%, while the hypophysis increased 66% (body weight 206 gm., ovaries 0.042 gm., hypophysis 0.016 gm.).

The histologic examination of the ovaries and anterior pituitary glands presented some interesting changes. The ovaries of the rats injected for 63 days showed the characteristic picture of small corpora lutea, lutein cysts, and developing graafian follicles. In 94 days they gave essentially the same findings, but the anterior hypophysis showed a marked increase in the number of basophilic cells with many vacuolated "castration cells". After 150 and 179 days the changes in the anterior lobe were present to a more marked degree, while the ovaries had undergone a pronounced atrophy, which was also noted in the uterus and vagina. The ovaries generally contained a few atretic follicles but no corpora lutea, and the stroma showed fibrous tissue growth with hyaline degeneration.

Pregnancy Blood Extract. The administration to female rats of a chorionic gonadotropic hormone prepared from blood of pregnant women resulted in an increase of ovarian weight without the subsequent marked diminution in size which has been reported from the use of similar extracts made from pregnancy urine or placentae (Zondek,[3] McPhail,[4] Collip *et al.*,[5] Wolfe[6]). During the first 175 days of this experiment a dilution of the extracts was used, which in 5 days induced an average increase of 130% in the weight of the ovaries of 20 immature rats, but subsequently the preparations were employed at a dosage which produced an average 5-day increase of 250% in 7 young rats. The average increase in ovarian weight of 11 rats injected for 28 days was 400% and in the weight of the hypophysis, 25% (body weight 75 gm., ovaries 0.071 gm., hypophysis 0.004 gm.); in 59 days the ovaries of 7 rats showed increases of 200% and the hypophysis 80% (body weight 103 gm., ovaries 0.137 gm., hypophysis 0.009 gm.); in 90 days, 9 rats gave an average increase in the weight of the ovaries of 289% and the hypophysis of 48% (body weight 152 gm., ovaries 0.240 gm., hypophysis 0.012 gm.); and in 193 days the ovaries had increased 343% in weight while the hypophysis gave the same average weight as the controls (body weight 188 gm., ovaries 0.232 gm., hypophysis 0.013 gm.). Four rats were injected daily for 272 days, and owing to the wide variations found in organ weights it seems more accurate to quote the gross weights of each animal instead of using the average for the whole group. The body weights were 235, 248, 232, and 251 gm., the ovaries 0.091, 0.312, 0.337, and 1.137 gm., and the hypophysis 0.018, 0.031, 0.033, and 0.018 gm., while 2 controls gave an average body weight of 204 gm., ovaries 0.066 gm., and the hypophysis 0.011 gm. The injections were continued for 365 days in 2 animals. One (No. 25) became ill toward the end of the experiment and weighed only 190 gm., while the ovaries weighed 0.205 gm., and the hypophysis 0.008 gm. The second rat (No. 37) weighed 253 gm., ovaries 0.236 gm., and hypophysis 0.034 gm., as compared to the average of 2 littermate controls with body weight 242 gm., ovaries 0.068 gm., and hypophysis 0.014 gm.

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[3] Zondek, B., *Die Hormone des Ovariums und des Hypophysenvorderlappens*, Julius Springer, Berlin, 1931.

[4] McPhail, M. K., *J. Physiol.*, 1933, **80**, 105.

[5] Collip, J. B., Selye, H., Thomson, D. L., and Williamson, J. E., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 590.

[6] Wolfe, J. M., *Anat. Rec.*, 1935, **63**, 1.

The histologic examination of the ovaries of the rats injected for 272 days or less showed the presence of developing follicles, lutein cysts, large corpora lutea, and a hypertrophy of the stromal cells. A characteristic finding was the occurrence of relatively large cysts lined with connective tissue, which increased in number as the experiment progressed. The ovaries of the rats injected for one year contained many such cysts, the stroma was atrophic, there was an extensive round-cell infiltration and many phagocytic cells containing blood pigment. The enlarged anterior lobes showed changes apparently comparable to those found by Severinghaus,[7] Wolfe *et al.*,[8] and Wolfe[6] following the injection of pregnancy urine, namely, an increase of chromophobes and a decrease in eosinophiles, but careful cell-counts were not conducted. The uteri of 2 rats injected for 272 and 365 days respectively showed an extensive metaplasia of cylindrical epithelium to squamous epithelium. The mucosa of the vaginae of rats treated for 193 days or longer underwent extensive vacuolization and the lumina were lined with tall, stratified, mucified cells.

Summary. The daily administration for periods of 2 to 6 months of a sheep anterior pituitary extract to female rats resulted in a marked atrophy of the ovaries after a preliminary increase in weight. This change was associated with an increase in the weight of the hypophysis and the appearance of many "castration cells" in the anterior lobe.

The continued daily injection of a gonadotropic preparation made from blood of pregnant women produced a marked increase in ovarian weight which was maintained for as long as one year. The hypophysis also showed a marked increase in weight which was associated with histologic changes affecting the chromophobes and eosinophilic cells.

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[7] Severinghaus, A. E., *Anat. Rec.*, 1934, **60**, 43.

[8] Wolfe, J. M., Ellison, E. T., and Rosenfeld, L., *Anat. Rec.*, 1934, **60**, 357.

8755 P

Cytological Studies of Biopsied Pernicious Anemia Bone Marrow During Relapse.

OLIVER P. JONES. (Introduced by C. M. Jackson.)

From the Hematological Laboratory, Department of Anatomy, University of Minnesota.

In the past we have reported that in pernicious anemia marrow during relapse there is a pathologic hemoglobiniferous series, the megaloblastic, which is not related to the normoblastic or definitive red cell series.[1] The only thing these two series have in common is their ancestral cell, the myeloblast. The present report deals with certain observations which were made while the above mentioned work was in progress and takes into consideration pathologically altered neutrophils and megakaryocytes found in pernicious anemia marrow.

The present investigation was carried out upon nine bone marrows from patients with pernicious anemia during relapse. Six were prepared by smearing freshly aspirated sternal marrow and three were dry imprints (abklatsch). All preparations were stained with May-Grünwald-Giemsa combination of Pappenheim. The best morphological detail of marrow cells was elicited by the dry imprint method, consequently these preparations were studied more intensively than films of aspirated marrow.

Studying large neutrophils (macropolycytes) revealed that their entire life history is quite different from that encountered in normal bone marrow. As early as the leukoblast stage the nucleus has assumed a polymorphic shape as indicated by peculiar protuberances and invaginations. During the promyelocyte stage the nucleus may be shaped like a giant "stab" form. In other words, nuclear development and maturation has preceded that of the cytoplasm to such an extent the large pathologic neutrophils have skipped myelocytes and metamyelocyte stages. This particular point has been previously mentioned by Tempka and Braun.[2] Generally the arrangement of chromatin is less compact than that of corresponding stages in the normal neutrophil. The cytoplasm of promyelocytes may contain either rarified areas of light hyaloplasm or distinct vacuoles. These cytoplasmic vacuoles tend to disappear as the pathologic neutrophils mature. When these cells have obtained their full complement of granules practically all the vacuoles have vanished. Besides these vacuoles in the cytoplasm there are holes in the nuclei. These commence as a single definitely circumscribed hole in the nuclear material which later tends to become drawn out into an irregularly shaped space. Single large holes are found in some myelocytes which give them the appearance of a "doughnut". Other pathologic neutrophils were found with three or more holes in the nucleus, each being separated from the other by a strand of chromatin material of varying thickness. It is by means of these holes in the nuclear material that the pathologic neutrophil ultimately attains the morphology of macropolycytes (II and III) described by Cooke.[3]

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[1] Jones, O. P., *Anat. Rec.* (Suppl.), 1934, **58**, 23; *ibid.*, 1935, **61**, 57.

[2] Tempka and Braun, *Folia Haematol.*, 1932, **48**, 355.

Tempka and Braun[2] have interpreted these findings to indicate characteristics of degenerative alterations of the neutrophil. Our interpretation is that they represent processes in the maturation and development of a pathologic neutrophilic series. It is by means of this precocious nuclear polymorphism along with the appearance of holes in the nuclear material that the bizarre-shaped nuclei of the pernicious anemia neutrophil (macropolycyte) develop. This finding agrees with the third hypothesis of Cooke's in which he assumes that "the polymorph is inherently abnormal owing to a defect in its parent, the hemocytoblast, or in its environment"[3]; for, it has been shown that these pathologic alterations commence as early as the leukoblast stage and can be traced through the subsequent promyelocyte and myelocyte stages to the pernicious anemia neutrophil of the circulating blood.

The megakaryocytic system is involved in some bone marrows, but not to the extent claimed by Tempka and Braun.[2] These authors found numerous megakaryocytic nuclei devoid of cytoplasm and believe this to be the result of the same process which effected the erythropoietic and leukopoietic systems. The type of pathologic megakaryocyte which we encountered was an intensely basophilic one with a polymorphic nucleus. The cytoplasm was devoid of azurophilic granules. Normally when the nucleus is polymorphic the cytoplasm contains azure granules and is not as basophilic as the pathologic ones. Here it seems that the nucleus is maturing while the cytoplasm shows no tendency to develop azure granules and form platelets The pathologic alteration of the megakaryocytes in some pernicious anemia bone marrows may account for the decrease in platelets occasionally found in cases of pernicious anemia.

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[3] Cooke, W. E., *J. Lab. and Clin. Med.*, 1934, **19**, 453.

From these observations upon the qualitative alterations of the marrow cells one must conclude that the lack of anti-pernicious anemia principle produces a panmyelopathy, in that three distinct cell lines are effected. The normoblastic or definitive series is inhibited while the pathologic red cell series, the megaloblastic, proliferates. The neutrophilic series is effected to the extent that there is developing in the marrow a pathologic series which gives rise to the abnormal pernicious anemia neutrophils (macropolycytes) of the peripheral blood. Also, in some cases the megakaryocytes are pathologically altered so that they are not producing platelets. This interpretation of the panmyelopathy in pernicious anemia is different from Tempka and Braun's[2] in that we do not believe the qualitative alterations in the neutrophils and megakaryocytes represent degenerative manifestations. The very fact that all of these above mentioned alterations disappear under adequate therapy and the marrow returns to normal favors our interpretation.[4]

8756 P

The Isolation of *Brucella abortus* from the Blood-Stream of Cattle.*

C. P. FITCH, LUCILLE M. BISHOP AND MARGARET D. KELLY.

From the University of Minnesota Agricultural Experiment Station, St. Paul.

Soule[1] has perhaps done the most extensive work on this subject. His method consisted of adding 50 cc. of whole blood to 450 cc. of glycerol-infusion-broth and then incubating under 10% carbon dioxide. More than 5000 ordinary "herd-run" cattle were studied. Two series of tests were made on each animal at intervals of approximately 6 months. In the first series of tests agglutinins were present in the blood of 2,237 of the animals but only 299 gave positive blood-cultures. In the second series agglutinins were present in 2,607 cases with 206 positive blood-cultures. There were 40 positive blood-cultures without concomitant agglutinins.

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[4] Tochowicz, L., *Folia Haematol.*, 1934, **53**, 16; Braun, B., *ibid.*, 1934, **53**, 27.

[*] Paper No. 1429 of the Journal Series of the Minnesota Agricultural Experiment Station.

[1] Soule, M. H., *Premier Congrés International de Microbiologie*, 1930, **1**, 606.

This cultural method evidently requires extreme aseptic precautions in order to prevent contamination of the cultures with extraneous organisms. In our use of this technic, contaminations with molds, spore-forming bacteria, and gram-positive cocci occurred in nearly two-thirds of our cultures and we isolated *Brucella abortus* but once in 75 attempts.

Because of these discouraging results we studied 4 other methods, using the blood of cattle and more extensively the blood of guinea pigs. Rainsford's[2] method of removing all the serum from a clot of blood and then culturing the clot was used as well as the technic of Boez and Robin.[3] The latter authors maintain that the bactericidal action of the blood is destroyed by mixing it with an acid citrate which prevents clotting and reduces the pH to approximately 5.5. This acidity will not kill the bacteria but does not allow their multiplication. The acidity is counteracted by cultivation in an alkaline medium at pH 8.3. The mixed blood and medium attain a pH of approximately 7.5 which is suitable for the growth of the majority of bacteria. Haring[4] has had success drawing blood into sufficient sodium citrate to prevent clotting, heating to 56°C. for 15 minutes and inoculating blood agar slants with the heated mixture. · Stewart *et al.*[5] recommend the method of Massa and Battistini[6]: The blood is collected in *liquoïde* "Roche" (sodium polyanetholsulphonate), which is anticoagulant and destructive of leukocytes but not bacteria. Ten cc. of blood are drawn into 2 cc. of 1% aqueous solution of *liquoïde*. The mixture is incubated for 10 days and then transplanted to agar slants. Continued incubation of the blood and *liquoïde* mixture may be allowed and subcultures made at intervals.

The blood of each of 12 cows with long-standing Bang's disease was tested at least once by the above 5 technics. *Brucella abortus* was isolated but once from 105 cultures; this isolation was made by the acid-citrate method.

A study was then made to determine if a bacteremia was present in the incubative stages of the disease. Two heifers in early pregnancy were artificially infected. Daily blood-cultures were made from these animals using Haring's heated citrate method. Table I shows the results of this study.

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[2] Rainsford, S. G., *J. Roy. Nav. M. Serv.*, 1933, **19**, 1.

[3] Boez, L., and Robin, L. H., *Compt. rend. Soc. de biol.*, 1929, **101**, 1009.

[4] Haring, C. M., personal communication.

[5] Stewart, B., *et al.*, *California and West. Med.*, 1935, **43**, No. 2.

[6] Massa, M., and Battistini, G., *Ztschr. für Bakt.*, 1 Abt., Originale, 1934, **131**, 241.

TABLE I.

Animal No.	Type and date of infection	Agglutinative titre of 1:100	*Brucella abortus* isolated on following dates
342	*Per os*, 1-28-36	3-2-36	Feb. 26, Mar. 2, 7, 11, 18, 24, 27, May 1
349	Conjunctival, 2-13-36	2-24-36	Feb. 24, Mar. 3, 4, 11, 28, April 28, May 5

Lübke[7] found a bacteremia in 90.4% of 21 recently infected animals and 5.9% of 81 cases of old infections. These studies were made by inoculating guinea pigs with 5 cc. of citrated blood and later culturing the guinea pigs. Direct cultures from the blood were not attempted. This inoculative method did not yield as good results in our hands as cultural methods. Cultures and inoculations were made in duplicate in 40 cases. *Brucella abortus* was never isolated by the inoculative method but was isolated 7 times by culture. On several occasions only 1 or 2 colonies of *Brucella* were found, indicating that relatively few bacteria were present in the blood. The numbers were probably not sufficient to infect the guinea pigs.

Supplementary work on cultural methods was done on the blood of guinea pigs. It was determined that a bacteremia occurs regularly 24-48 hours after intra-abdominal inoculations with *Brucella abortus*. Cardiac punctures were made at this time on 33 of the guinea pigs. Six cc. of blood were drawn from the heart and used in 1 cc. amounts to compare the above technics. *Brucella abortus* was isolated from 27 of these samples by one or more methods. It was isolated 12 times from the clot, 15 times from the whole-blood infusion-broth, 17 times from the acid citrate, 18 times from the heated citrate, and 21 times from the *liquoïde* cultures.

These results indicate that a bacteremia is not common in cattle with long-standing Bang's disease, but may be easily detected in recently infected cattle; also that the acid citrate, the heated citrate and the *liquoïde* culture methods are efficient for detecting a bacteremia due to *Brucella abortus*.

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[7] Lübke, A., *Ztschr. f. Infektionskr.*, 1935, **47**, 240.

8757 P

Sensory Nerve Terminations Associated with Peripheral Blood Vessels.

Raymond C. Truex. (Introduced by Albert Kuntz.)
From the St. Louis University School of Medicine.

The end organs in the walls of the arteries and veins and perivascular tissue in the forelimb of the cat, which are connected with afferent nerve fibers, have been studied with the aid of a modified pyridine-silver technique in preparations in which the sympathetic nerve fibers had undergone degeneration following extirpation of the inferior cervical sympathetic ganglion. Eight cats were subjected to this operation under aseptic conditions and allowed to live 46 to 81 days in order to insure complete degeneration of the sympathetic fibers supplying the vessels in question.

The end organs observed in the perivascular tissue include delicate skein-like structures made up of the terminal branches of slender nerve fibers, groups of cells aggregated in fatty interspaces, and arborizations of coarser nerve fibers the terminal branches of which form brush-like structures which ultimately end freely or in relation to small rounded cells. The fibers connected with these end organs deviate from nerves in the adventitia.

The end organs in the adventitia are coarser than those in the perivascular tissue. Those observed in the proximal portions of the larger arteries are connected with myelinated fibers which divide into short stout processes and end as dark club-shaped knobs. Similar structures with finer and more diffuse terminations were also observed.

The endings in the adventitia of the distal muscular arteries are neither as thick nor as extensive as those observed in the larger arteries and the perivascular tissues, and are confined to the middle or deeper zones. They lie in relation to small refractive cells and are of 3 types: ramifying myelinated fibers with unmyelinated terminal branches; segmented structures adjacent to the muscular coat, and tortuous individual fibers which end in relation to single falciform cells.

No specialized nerve-endings were observed in the media. Unmyelinated afferent nerve fibers enter this layer in slender bundles after a spiral course parallel to the longitudinal axis of the vessel, and after a tortuous course end freely in the connective tissue between the smooth muscle cells.

Afferent nerve fibers to veins have been observed only in the adventitia, where they run a straight course without extensive plexus formation. Sensory end-organs connected with these fibers have been observed in the larger veins, but not in the distal smaller ones.

Some of the earlier investigators have described delicate nerve fibers, presumably afferent in character, which terminate in the intima. No nerve fibers could be observed in the intima in any of our preparations. No ganglion cells were observed along any peripheral vessels.

The afferent end organs described above are morphologically similar to certain of those described by Sunder-Plassman (1930)[1] in the carotid sinus and those described by Larsell (1921, 1922, 1935)[2] and Larsell and Dow (1933)[3] in the respiratory tract.

8758 C

Coexistence of Nuclear Inclusions in Salivary Glands and Kidneys of Wild Rats.*

L. E. RECTOR.

From the Anatomical Laboratory, Washington University, St. Louis.

The purposes of this study were: (1) To ascertain whether intranuclear inclusions, similar to those found in wild rats in England[1, 2] and China,[3, 4] occur also in the United States. (2) To discover whether, if present, they are restricted to salivary glands as has been described.[3] (3) To demonstrate whether a virus, responsible for their formation, is transmissible to laboratory rats.

A series of 120 wild rats was collected at 16 different locations in St. Louis. All were trapped or shot on rubbish heaps, except 8 which were captured at a meat packing plant; and all were brown

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[1] Sunder-Plassman, Paul, *Zeitschr. f. Anat. und Entwg.*, 1930, **93**, 567.

[2] Larsell, O., *J. Comp. Neur.*, 1921, **33**, 105; *ibid.*, 1922, **35**, 97; *ibid.*, 1935, **61**, 407.

[3] Larsell, O., and Dow, R. S., *Am. J. Anat.*, 1933, **52**, 125.

* Aided by a grant from the Rockefeller Foundation for research on virus diseases.

[1] Hindle, E., and Stevenson, A. C., *Tr. Roy. Soc. Trop. Med. and Hyg.*, 1929-30, **23**, 327.

[2] Hindle, E., *Nature*, 1932, **129**, 796.

[3] Kuttner, A. G., and Wang, S. H., *J. Exp. Med.*, 1934, **10**, 773.

[4] Kuttner, A. G., and T'ung, S., *J. Exp. Med.*, 1935, **62**, 805.

rats *(Rattus norvegicus)* except 2 which were black *(Rattus rattus)*. Their weights ranged from 45 to 541 gm. with an average of 300.2 gm. There were 50 males and 70 females. The parasites found in 100 members of the series have been described.[5] Tissues were fixed in Zenker's fluid and stained in hematoxylin and eosin. Care was taken to examine all of them with as nearly as possible equal thoroughness.

Nuclear inclusions were found in both the submaxillary glands and kidneys of 24, and in the kidneys alone of 87 of these wild rats. Evidently, therefore, the limitation to the salivary glands reported by Kuttner and Wang[4] for Chinese wild rats did not hold for wild rats in the St. Louis area, but these investigators showed that, when white rats were injected with salivary glands containing inclusions, inclusions were formed in their kidneys. This indicates that a single virus may be responsible in this series for the formation of the nuclear inclusions in both salivary glands and kidneys.

Examination failed to reveal the natural occurrence of nuclear inclusions in the submaxillaries and kidneys of 50 white rats in our colony. An attempt was made to transmit a virus from wild rats to white rats. The kidneys of 2 wild rats, proved to contain typical inclusions, were emulsified in sterile salt solution and passed through a Berkefeld N filter. Four lots, each of 2 rats, were given intraperitoneally 1 cc., subcutaneously 1 cc., intraglandularly (submaxillary) 1 cc., and intracerebrally 0.5 cc., respectively, of the filtrate. They were killed at intervals up to the 20th day but no inclusions could be found in salivary glands, kidneys or nervous system.

The inclusions in the submaxillaries of these wild rats resemble those previously reported in other species[6-10] but they differ from the inclusions described and figured by Thompson[8] in the salivary glands of white rats; which are not surrounded by similar unstained areas and are smaller. They were, moreover, absent in rats of less than 245 gm., whereas Thompson found inclusions only in young rats and not in 12 rats 6 months old. The smallest rat exhibiting renal inclusions weighed 102 gm. and only 3 rats over 100 gm. failed to show them. It is likely that the age of virus invasion, under the conditions prevailing in this series, is fairly definite and that it usually attacks the kidneys first and then spreads to the submaxillary glands in some animals. The virus appears to be quite benign, because there were no accompanying infiltrative or degenerative changes in the kidneys, and, in the submaxillary glands, such alterations were more often absent than present. Measurements of the inclusions reveal considerable uniformity in size which is thought[11, 12] to indicate a static rather than an active process. Whether this virus in wild rats is capable of infecting humans is an open question although inapparent salivary gland viruses are remarkably species specific. It has been shown, however, that a virus ordinarily restricted to the salivary glands under certain conditions may be induced to extend widely through the body.[13]

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[5] Tsuchiya, H., and Rector, L. E., *J. Parasit.*, 1935, **21**, 426.

[6] Ribbert, H., *Centralbl. f. allg. Pathol. u. path. Anat.*, 1904, **15**, 945.

[7] Jackson, L., *J. Inf. Dis.*, 1920, **26**, 347.

[8] Thompson, M. J., *J. Inf. Dis.*, 1932, **50**, 162.

[9] Rector, L. E. and E. J., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 192.

[10] Thompson, J., *Am. J. Path.*, 1934, **10**, 676.

8759 P

Mechanism of Formation of Hexosemonophosphate in Muscle and Isolation of a New Phosphate Ester.

C. F. CORI AND G. T. CORI.

From the Department of Pharmacology, Washington University, School of Medicine, St. Louis.

Experiments performed on intact frog muscle indicated that hexosemonophosphate, in contrast to hexosediphosphate, is formed by esterification with inorganic phosphate.[1] A further study of this problem was carried out on minced frog muscle which was almost completely inactivated (in regard to lactic acid formation) by 3 to 4 extractions with distilled water. When such muscle, which contains only 2 to 4 mg. % of organic, acid-soluble P, is incubated anaerobically for 3 hours in isotonic phosphate buffer, the organic P content rises to 8 to 13 mg. % due to the formation of hexosemonophosphate. Addition of small amounts of adenylpyrophosphoric or of adenylic acid greatly enhances the formation of hexosemonophosphate, as shown in Table I . The experiments indicate that hexosemonophosphate is formed from inorganic phosphate and that adenylic acid serves as the mediator of this reaction.

Observations after short periods of incubation showed that the first phosphorylation product is not hexose-6-phosphoric acid

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[11] Rector, E. J. and L. E., *Am. J. Path.*, 1934, **10**, 629.

[12] Cowdry, E. V., and Scott, G. H., *Am. J. Path.*, 1935, **11**, 647.

[13] McCordock, H. A., and Smith, M. G., *J. Exp. Med.*, 1936, **63**, 303.

[1] Cori, G. T., and Cori, C. F., Summaries of Communications, XVth International Physiological Congress, p. 66, 1935.

TABLE I.

Effect of Coenzyme on Hexosemonophosphate Formation in Inactivated Muscle.

Minced muscle was extracted 3 times with 20 times its weight of distilled water and was incubated anaerobically in isotonic phosphate buffer (pH 7.2) at 20° for 3 hours.

Values in mg. per 100 gm. muscle.

Hexosemonophosphate				
hexose	P found	P calc.	Lactic acid	Additions (per 100 gm. muscle)
15		3	6	Incubated in isotonic KCl
68	10	12	8	
162	32	28	30	6 mg. adenylpyrophosphate P
75	11	13	10	
141	36	24	25	6 mg. adenylpyrophosphate P
68	13	12	10	
264	59	46	26	12 mg. adenylic acid P
48	8	8	10	
119	28	21	12	4 ” ” ” ”

(Embden ester), but a new ester which is slowly converted to the 6-phosphoric acid under the conditions of these experiments. (Table II.) After one and 2 hours of incubation much more organic P is present than can be accounted for on the basis of reducing power, assuming the latter to be due to hexose-6-monophosphate. Hydrolysis in N H_2SO_4 at 100° for 10 minutes revealed the presence of a compound which (unlike the 6-monophosphoric acid) is easily hydrolyzed in acid and which yields equivalent amounts of fermentable sugar and inorganic phosphate.

The new compound was isolated from 4 different preparations. About 100 gm. of minced and washed muscle, to which 120 mg. of

TABLE II.

Effect of Length of Time of Incubation on Phosphorylation.

Values in mg. per 100 gm. muscle.

	Hexosemonophosphate fraction			
Time hr.	hexose	P found	P calc.	Additions (per 100 gm. muscle)
0	5		0.9	
1	79	62	14	13 mg. adenylic acid P
2	131	69	23	” ” ” ” ”
3	252	67	43	” ” ” ” ”

After 10 min. hydrolysis in N H_2SO_4.

		Fermentable Sugar	
	Inorg. P	found	calc.
1	39	219	226
2	45	256	261
3	20	122	116

adenylic acid had been added, was incubated for one hour in phosphate buffer. After deproteinization with $HgCl_2$, the water-soluble barium salts were precipitated with alcohol. The soluble barium salts consisted of about 65% of the new compound, the remainder being the adenylic acid added and the small amount of hexose-6-phosphoric acid formed. Fractionation of the barium salts was not successful and they were therefore converted to the brucin salts. After removal of most of the adenylic acid by concentrating the aqueous solution of the brucin salts to a small volume, the remainder of the brucin salts was evaporated to dryness. The semi-crystalline residue was extracted with hot methyl alcohol containing 1% ethyl alcohol. On cooling the ester crystallized out in the form of fine needles which soon formed large aggregates. After recrystallization from methyl alcohol, the brucin salt was reconverted to the barium salt and purified further by repeated solutions in water and precipitations with alcohol. The final product contained 1.5% of an ester difficult to hydrolyze in acid as judged by the ratio of easily hydrolyzable to total organic P.

Elementary analyses for C, H, P, and Ba and determination of the sugar formed after hydrolysis (by means of hypoiodite) agreed with a compound of the composition of $C_6H_{11}O_5.PO_4Ba.3H_2O$. $[\alpha]^{25}_{D} = 75.5°$ (calc. for the anhydrous barium salt in water; conc. 1.26%). During hydrolysis in 0.01 N HCl at 70°, sugar and inorganic phosphate were liberated at the same rate, the percentages being 21.1, 38.3, 62.0 and 76.3 for the 30, 60, 120, and 180 minute periods, respectively. The percentages calculated for a velocity constant of 3.47×10^{-3} were 21.4, 38.1, 61.9, and 76.2, respectively.

The ester (when unhydrolyzed) does not reduce alkaline copper solution and does not react with hypoiodite, and it can be heated to 130° without discoloration, all factors which point to the absence of a free reducing group. We believe that the new ester is an aldose-1-phosphoric acid, the sugar being presumably glucose.

When the new ester is added to frog muscle extract, it is converted in a few minutes to an ester difficult to hydrolyze in acid and possessing reducing power, presumably hexose-6-phosphoric acid. The same change occurs in muscle extract inactivated by dialysis, so that the presence of the coenzyme system does not appear to be necessary for the wandering of the phosphate group.

Summary. In minced and washed frog muscle incubated in phosphate buffer, added adenylic acid transfers inorganic phosphate to carbohydrate resulting in the formation of hexosemonophosphate. The first phosphorylation product proved to be a new ester which

was isolated as the crystalline brucin salt and had the properties of glucose-1-phosphoric acid; when added to frog muscle extract it was converted in a few minutes to the Embden ester.

8760 P

Effect of Excessive Dietary Sodium Chloride upon Liver and Muscle Glycogen in the Rat.

D. GLEN CRABTREE AND BERNARD B. LONGWELL. (Introduced by Robert C. Lewis.)

From the Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado.

Chaikelis,[1] in 1934, stated that the inverse ratio between blood chlorides and blood sugar was not a compensatory mechanism concerned with the maintenance of proper osmotic pressure relations. He suggested that the blood chloride change was associated with some phase of carbohydrate metabolism. Following his report an investigation was started in this laboratory to determine what effect variations in the amount of sodium chloride in the diet would have upon the deposition of glycogen in the liver and muscles of the white rat.

Methods. Series 1. Young male rats were divided into 18 groups consisting of three animals each, the majority of which did not vary in weight by more than 10 gm. One animal of each group was placed on a diet poor in chlorides, one was placed on a diet rich in chlorides, and the third received a control diet. The control diet consisted of casein 33 percent, agar agar 2 percent, Osborne and Mendel[2] salt mixture 4 percent, cod liver oil 2 percent, butter (unsalted) 7 percent, yeast 8 percent, and sucrose 44 percent. The chloride poor diet was of the same composition as the control diet except that the Osborne and Mendel "chloride free" salt mixture[2] was substituted for the complete salt mixture which was used in the control diet. The chloride rich diet consisted of a food mixture of the same composition as the chloride poor diet with the addition of NaCl to a level of 6.25 percent of the total diet. Distilled water was given *ad libitum.*

The animals were kept in individual cages over wire screens. The temperature of the room was controlled so that it did not vary from 75°F. by more than 4 degrees.

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[1] Chaikelis, A. S., *J. Biol. Chem.*, 1934, **105**, 767.

[2] Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1918, **34**, 131.

The diets described above were fed for 10 to 15 days, and the experimental period was terminated by a 24-hour fast, at the end of which time the animals were anesthetized with sodium amytal. The gastrocnemius muscle was exposed and frozen *in situ* by means of a mixture of solid CO_2 and ether, and the frozen muscle was removed and immersed in this mixture. The liver was then removed and frozen immediately in the same manner. The tissues were weighed in the frozen state and analyzed for their glycogen content by the method of Good, Kramer and Somogyi.[3] The glycogen hydrolysates were analyzed for their glucose content by the method of Shaffer and Somogyi.[4]

Series 2. Using the same technic, a second series of experiments was carried out upon 20 groups of three animals each. The liver glycogen only was determined in this series. The animals were maintained upon the experimental diets for 22 to 30 days. The diets were identical with those used in Series 1 with the exception that the chloride rich diet contained 9.09 percent NaCl.

TABLE I.
The Effect of Variations in Dietary NaCl upon Liver and Muscle Glycogen.

	Series 1*		Series 2†
Diet	Liver glycogen (gm./100 gm.)	Muscle glycogen (gm./100 gm.)	Liver glycogen (gm./100 gm.)
Control diet	0.18 ± .01	0.29 ± .005	0.30 ± .03
Chloride poor diet	0.19 ± .02	0.32 ± .007	0.37 ± .04
Chloride rich diet	0.31 ± .03	0.31 ± .010	0.75 ± .03

*Series 1. The chloride rich diet contained 6.25% NaCl.
†Series 2. The chloride rich diet contained 9.09% NaCl.

Results. The results of these experiments are given in Table I. In order to conserve space we are not reporting the glycogen values of individual animals at this time; therefore, only the mean values and the probable error of each mean are given. In both series of experiments the rats which received a diet rich in sodium chloride showed a large increase in liver glycogen. The chloride poor diet did not have any significant effect upon the liver glycogen stores. The results obtained from the muscle glycogen determinations showed that the mean values obtained from the groups on the chloride poor and chloride rich diets did not vary strikingly from the mean value obtained for those on the control diet. This, however, is being reinvestigated at the present time.

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[3] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

[4] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

We have made no studies to ascertain the mechanism by which excessive amounts of sodium chloride in the diet cause an increase in liver glycogen stores, but we plan to do so in the future. A study is now in progress to determine whether the sodium ion, the chloride ion or a combination of the two is responsible for the results obtained.

These results are of considerable interest in connection with the observation of McQuarrie and coworkers[5, 6] that when large amounts of sodium chloride were added to the diet of diabetic children the fasting blood sugar was lowered and glycosuria was decreased.

8761 P

Volume Changes of Smooth Muscles of Phascolosoma During Activity.

Ernst Fischer.

From the Marine Biological Laboratory, Woods Hole.

Meyerhof's method[1] of measuring the volume changes of amphibian muscles was applied to the smooth muscles of Phascolosoma, a marine worm, belonging to the Sipunculidea. Using the retractor muscle in the volume chamber, after single or tetanic stimulation, a temporary change in volume was observed, starting almost simultaneously with the isometric mechanogram. However, only in a few of the experiments was the course of the volume curves more or less parallel to the tension curves. Often the volume curves showed 2 phases; sometimes even 3 were present. The direction of the volume changes varied greatly from experiment to experiment; but in general there was a tendency towards volume increase. The maximum change occurring in the course of the volume curves was, on the average, of the order of magnitude of 0,000 005 of the total muscle volume. The dependence of direction of the volume change on the initial length of the muscles could not be demonstrated so clearly for the Phascolosoma retractor as for amphibian muscle

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[5] Thompson, W. H., and McQuarrie, I., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 907.

[6] McQuarrie, I., Thompson, W. H., and Anderson, J. A., *J. Nutrition*, 1936, **11**, 77.

[1] Meyerhof, O., u. Hartmann, H., *Pflüg. Arch. f. d. ges. Physiol.*, 1934, **234**, 722; Meyerhof, O., u. Möhle, O., *Biochem. Ztschr.*, 1936, **284**, 1.

and turtle heart muscle as shown in a previous paper.[2] No distinct volume changes were observed during the recovery.

Pieces of the body wall, containing the longitudinal muscle fibers, showed spontaneous volume changes of about 0,000 02 of the total muscle volume, although the motor activity of the muscle was so weak that it could not be recorded with the isometric levers at my disposal.

Since the retractor muscle develops a smaller tension than a striated muscle, but has a volume change about as large as that of a striated muscle, a markedly higher volume change occurs in the retractor than in frog muscle per unit of tension developed. For the body wall muscles of Phascolosoma the volume change per gm. tension must be at least 100 times greater than for a frog sartorius. Meyerhof[1] assumes that the volume change is caused by the chemical processes involved in the energy output for contraction, and that the size of the volume changes is more or less parallel to the working metabolism. Since it is generally assumed that less energy is needed for the activity of smooth muscle than for the contraction of striated muscle, it is hard to explain why the volume change in smooth muscle is so much larger. Furthermore, the fact that the direction of the volume changes is not constant and can be influenced to a certain extent by the physical conditions (initial length or tension) indicates that other factors are much more important as the cause of the volume changes than the working metabolism of the muscles.

8762 C

Permeability to Water and Staining Rate of Developing and Resting Sea Urchin Eggs.*

D. M. WHITAKER.

From the Hopkins Marine Station, Stanford University.

It was shown some years ago by R. S. Lillie[1] that fertilized *Arbacia* (sea urchin) eggs are more permeable (about four times) to water than unfertilized eggs, since they swell and shrink more

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[2] Fischer, E., *Am. J. Physiol.*, 1935, **113**, 42.

* This work has been supported in part by funds granted by the Rockefeller Foundation. The author is indebted to Mr. Edward Lowrance for assistance in carrying out the experiments.

[1] Lillie, R. S., *Am. J. Physiol.*, 1916, **40**, 249.

rapidly in hypotonic and hypertonic sea water. The change in permeability to water at fertilization thus more or less parallels the change in rate of oxygen consumption. It had also been shown earlier by Lyon and Shackell[2] that at fertilization the egg of *Lytechinus* (sea urchin) increases its permeability to certain dyes but not to others. The immature eggs were found to be even more permeable to certain dyes than the fertilized egg, showing that maturation is associated with a decrease in permeability and that low permeability to dye thus correlates with the absence of cell division in the unfertilized egg.

Since the unfertilized egg is in a state of developmental inhibition, from which it is released at fertilization, it is of some interest to examine further the properties of the developing immature egg, before it enters the state of inhibition, for comparison with the developing fertilized egg.

A number of experiments on the eggs of the sea urchin *Strongylocentrotus franciscanus* show that immature eggs, like fertilized eggs, stain deeply much more rapidly in sea water containing Methylene Blue, Gentian Violet, or Methyl Violet than do unfertilized resting eggs. This is true in dilutions of the dyes in which the first cell division takes place as well as in stronger concentrations which are more quickly toxic. The phenomenon may be demonstrated more accurately if the jelly is removed since this may stain heavily. In this species as in *Lytechinus* the immature eggs appear to stain somewhat more rapidly than the fertilized eggs. These results show almost beyond doubt that the permeability to these dyes is much less in the resting unfertilized stage, although it must be granted that the effect of the biochemical state of the cell on entered dye may also be involved. The three types of eggs of the sand dollar *Dendraster excentricus* show essentially the same relations to the three dyes.

The permeability to water of the three types of eggs of *S. franciscanus* was investigated in some 16 series of measurements. In each series, an egg of each type which was very nearly circular as seen from above was selected from the same female, and the three types were selected with the same initial diameter in sea water. This was done by selecting unfertilized and fertilized eggs which were slightly smaller than average. By means of a micro-lip-pipette it was possible to transfer an egg with a very small amount of sea water into dilute (50%) sea water and suck new medium about it in about 10 seconds. The diameter was periodically measured as swelling progressed. The cells were presumably very nearly spherical, but since no side view observations were made to check against flattening, the results are given in terms of diameters as seen from above without attempting to convert to volumes. Sev-

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[2] Lyon, E. P., and Shackell, L. F., *Science*, 1910, **32**, 249.

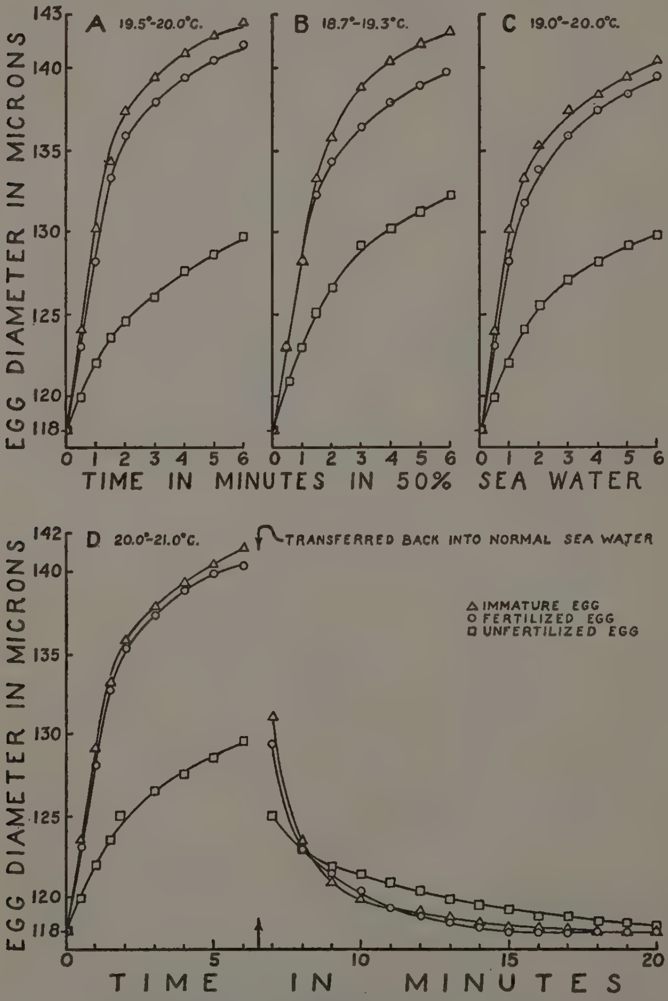

FIG. 1.

Rate of swelling of immature, unfertilized, and fertilized eggs of *S. franciscanus* in dilute (50%) sea water (A. B. C.). Also shrinking after return to normal sea water (D).

eral typical results are shown in Fig. 1. All results indicate that immature eggs, like fertilized eggs, swell more rapidly than the unfertilized eggs. Under the conditions of the experiments in 50% sea water the immature eggs usually swelled slightly more rapidly than the fertilized eggs, and the equilibrium diameter was usually somewhat greater.

In 7 series the eggs were returned to normal sea water after swelling for 6 minutes in dilute sea water. A typical result is shown in Fig. 1D. These 7 experiments indicate that in exosmosis as in endosmosis the permeability to water of the immature egg as well as of the fertilized egg is considerably greater than the permeability of the resting unfertilized egg.

The immature eggs used throughout this work were almost as large as the average mature egg, but they were entirely immature since the large germinal vesicle was present well within the cell. If the germinal vesicle is too close to the surface (in eggs on the verge of maturation) it is apt to burst under osmotic stress after which the egg is often fertilizable. Such border line types of eggs were avoided. Complete maturation normally precedes the resting stage in the sea urchin egg.

Eggs were used from 9 different females of *S. franciscanus* in April and May, 1936. At the start of swelling, the various immature eggs had been in sea water 20 minutes to 2 hours, the unfertilized eggs 20 minutes to 2.5 hours, and the fertilized eggs 35 minutes to 3 hours. In all cases the fertilized eggs were placed in dilute sea water at approximately the same developmental stage, namely, when the hyaloplasmic layer was first definitely secreted. Depending on temperature, this was of the order of 10 minutes after fertilization. Temperature ranged from 17°C. to 22°C. over all, but did not vary more than 2°C. within a series.

It has been shown by Goldforb[3] that the permeability of unfertilized *Arbacia* eggs increases as they age, and by Lillie[4] that the permeability of the fertilized eggs varies initially with time after fertilization.[5] The present results do not cover these variables and therefore no attempt is made to show the exact ratios of the permeabilities of the three types of eggs. It is rather the orders of magnitude which are indicated, with relative similarity between immature and fertilized eggs under the conditions of the experiments.

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[3] Goldforb, A. J., *J. Gen. Physiol.*, 1935, **19**, 149.

[4] Lillie, R. S., *Am. J. Physiol.*, 1918, **45**, 406.

[5] See also Dorfman, W. A., *Protoplasma*, 1932, **16**, 56.

Summary and Conclusions. Immature eggs of the sea urchin *S. franciscanus,* like fertilized eggs, stain more rapidly in solutions of Methylene Blue, Gentian Violet, and Methyl Violet than do resting unfertilized eggs. Like the fertilized eggs they are also more permeable to water. The immature and fertilized eggs are developing while the unfertilized egg is in a state of developmental inhibition. Increase in water permeability, as well as staining rate, at fertilization (when the egg is released from inhibition) is thus the converse (in direction) of the change when the immature egg enters the resting stage.

8763 P

Concentration of Gonadotropic Substance from Pregnancy Urine.*

H. L. FEVOLD AND F. L. HISAW.

From the Biological Laboratories, Harvard University.

Katzman and Doisy[1, 2] reported that the gonadotropic substance found in the urine of pregnant women (P.U.) can be concentrated conveniently by adsorption on benzoic acid or by precipitation with phosphotungstic acid. Hellbaum *et al.*[3] have shown that the same substance is precipitated quantitatively from the urine by tannic acid. However, the purification of the tannic acid precipitate is rather difficult.

We have found that cresol extracts P.U. efficiently and its use offers definite advantages over the methods mentioned in simplicity, ease of manipulation and recovery of reagents. It removes the active material from the urine as completely as does tannic or benzoic acid and the product is a powder which is a convenient starting material for further purification.

The urine used in these experiments was from a stock supply which had been collected and kept in the coldroom for several days. The cresol was the U.S.P. grade of J. T. Baker Co.

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* Aided by a grant from the National Research Council, Committee on Problems of Sex.

[1] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1932, **98**, 739.

[2] Katzman, P. A., and Doisy, E. A., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1188.

[3] Hellbaum, A. A., Fevold, H. L., and Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1566.

The routine extraction of pregnancy urine is carried out as follows: The urine, in a separatory funnel, is shaken with 1/10 volume of cresol. After allowing to separate, the cresol is drawn off and the urine is extracted a second time with 1/10 volume cresol. The second portion of cresol is then used for the first extraction of a fresh batch of urine. The second urine sample is extracted a second time with a fresh batch of cresol which is also used for the first extraction of a third sample of urine, etc. The cresol, containing the active material is diluted with 2 volumes of acetone which precipitates the active substance. The precipitate is collected by centrifugation or by allowing it to settle to the bottom of the flask and the acetone-cresol solution decanted. The precipitate is washed with acetone and ether and stored as a dry powder. Twenty liters of urine, extracted in this manner yielded 12.6 gm. of dry powder which contained 1 rat unit per milligram. Another batch of urine (24 liters) yielded 19.4 gm. of powder which assayed 1 rat unit per 1.5 mg.

A series of experiments were carried out on the same batch of urine to determine the efficiency of extraction, as compared with other methods. One liter of urine was extracted three times with 100 cc. portions of cresol. The adsorbate was prepared as described and taken up in water for assay. Another liter of urine was treated once with 100 cc. cresol and prepared for assay as before. A third liter was precipitated with tannic acid and the crude tannate assayed. A fourth liter was extracted according to the benzoic acid method of Katzman and Doisy. The physiological activities of the 4 preparations were determined by injecting 22-day-old female rats twice daily for 3 days and weighing the ovaries the morning of the fifth day. The rat unit employed was the minimum amount which elicited a 50% increase in the weight of the ovaries. (Table I.)

TABLE I.
Assay of P.U. Preparations.

Method	R.U. per liter
Cresol (3x)	600-750
Cresol (1)	600-750
Tannic Acid	600-750
Benzoic Acid	500-600

Preliminary recovery experiments demonstrated that cresol extracts P.U. quite quantitatively from relatively dilute solutions. Two liters of urine were extracted 5 times with 1/10 volume of cresol. One liter of the urine was then boiled for 2 hours. The second liter was not boiled. One hundred milligrams of P.U. powder, equivalent to 50 R.U. were added to the extracted boiled urine and a similar amount to the extracted unboiled urine. Both samples were extracted twice with 1/10 volume of cresol in the usual manner. From the extracted boiled urine, 450 mg. of powder were obtained which contained 1 R.U. per 7 to 8 mg., indicating a recovery of 50 to 65 R.U. The extracted unboiled urine yielded a powder weighing 230 mg. which contained 1 R.U. per 5 to 6 mg., a recovery of 40 to 50 R.U. Both of these results would indicate a quantitative recovery as they are well within the limits of error for the assay method.

One hundred cubic cm. of pregnancy urine, containing 75 R.U. were diluted with 900 cc. of urine which had been extracted and boiled as described and the whole was extracted twice with 100 cc. cresol. The dried adsorbate weighed 330 mg. and contained 1 R.U. in 5 to 6 mg., representing a recovery of 55 to 66 R.U.

Cresol extraction has also been used in attempts to concentrate the follicle stimulating hormone from the urine of castrated women and women past the menopause (F.S.U.) but without success. While cresol is apparently very efficient in extracting P.U. it does not take up F.S.U. This demonstrates a striking difference in the properties of the 2 substances, at least as they exist in the 2 types of urine.

8764 P

Action of Crustacean Eye-Stalk Extract on Melanophores of Hypophysectomized Fishes, Amphibians, and Reptiles.*

A. A. ABRAMOWITZ. (Introduced by F. L. Hisaw.)

From the Biological Laboratories, Harvard University.

The discovery of the crustacean eye-stalk hormone by Perkins[1] and by Koller[2] was soon followed by announcements of its effects upon the chromatophores of vertebrates. Koller and Meyer,[3] Meyer,[4] Perkins and Kropp,[5] and Kropp and Perkins[6] reported contraction of fish melanophores following injections of eye-stalk extracts, and Kropp[5] and Kropp and Perkins[6] found melanophore expansion in tadpoles following similar injections. These divergent results led the writer to reinvestigate the physiological properties of eye-stalk extract with more rigidly-controlled, experimental animals. Extracts were made from the eye-stalks of *Palaemonetes vulgaris* and injected in various dosages into specimens of fishes, amphibians, and reptiles. The extracts were prepared by macerating eye-stalks in water. The supernatant liquid was boiled, filtered and dried. The dried material was extracted with hot 95% alcohol, the soluble fraction dried, washed with ether, and the ether insoluble material re-extracted with absolute ethanol. The soluble portion was taken up in water and injected.

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* Aided in part by a grant from the National Research Council Committee on Problems of Sex, administered by F. L. Hisaw.

[1] Perkins, E. B., *J. Exp. Zoöl.*, 1928, **50**, 71.

[2] Koller, G., *Zeitschr. f. vergl. physiol.*, 1928, **8**, 601.

[3] Koller, G., and Meyer, E., *Biol. Zentralbl.*, 1930, **50**, 579.

[4] Meyer, E., *Zool. Jahrb., Abt. Allg. Zool. u. Physiol.*, 1931, **49**, 231.

[5] Perkins, E. B., and Kropp, B., *Bio. Bull.*, 1932, **63**, 108.

[6] Kropp, B., and Perkins, E. B., *Bio. Bull.*, 1933, **64**, 226.

1. *Amphibian (Rana pipiens).* Completely hypophysectomized adult frogs, hypophysectomized tadpoles, normal white-adapted frogs and tadpoles, and normal black-adapted frogs and tadpoles constituted the experimental animals. Injection of eye-stalk extracts produced melanophore expansion in the normal white-adapted frogs and tadpoles, and in the hypophysectomized frogs and tadpoles, but had no effect on the melanophores of the black-adapted frogs and tadpoles.

2. *Fish (Ameiurus nebulosus).* Injection of eye-stalk extracts evoked melanophore expansion in white-adapted hypophysectomized catfishes, and in normal white-adapted catfishes; furthered the expansion of the melanophores of black-adapted hypophysectomized catfishes, and was without effect in normal black-adapted animals.

3. *Reptile (Anolis carolinensis).* Injected extracts evoked a darkening of normal white-adapted and hypophysectomized Anolis, but produced no change in the coloration of black-adapted specimens.

4. *Erythrophores of the Dace (Chrosomus erythrogaster).* Injection of eye-stalk extracts was followed by melanophore and erythrophore expansion in the dace.

The eye-stalk extract, it seems, expands the melanophores not only of an amphibian, as has been reported, but also expands those of a fish and a reptile, and has a similar effect upon the erythrophores of the dace. The writer has shown in a previous publication[7] that the effect of the ablation of the eye-stalks of Portunid crabs of Bermuda upon the black pigment of the carapace was similar to the reported effects of hypophysectomy upon the melanophores of some reptiles, lower fishes, and amphibians. The experiments reported here seem to add further support to the opinion already expressed, namely, that the physiological effects of the crustacean pigmentary hormone and those of the chromatophorotropic hormone of the intermediate lobe of the pituitary of vertebrates are identical with respect to the activity of the black pigment cells. This opinion, however, does not imply that the hormones, which at the present time have been obtained only in extract form, are chemically identical. Nevertheless, a comparison of the chemical properties of the eye-stalk hormone (or hormones) described in the papers of Kropp and his associates (including some additional data on the solubility of the active material in various organic solvents made by the writer) with those listed by Zondek[8] for intermedin reveals several interesting similarities.

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[7] Abramowitz, A. A., *Proc. Nat. Acad. Sci.*, 1935, **21**, 678.

8765 P

Absorption through the Nasal Mucosa of Tannic-Acid Treated Mice.

GEOFFREY RAKE AND HERALD R. COX.

From the Laboratories of The Rockefeller Institute for Medical Research, New York.

Reports have been given of the absorption of Prussian blue through the nasal mucosa of rabbits[1] and mice.[2, 3] A study by Olitsky and Cox[2] of the absorption of the pigment 1 to 3 hours after its instillation in mice previously treated intranasally with tannic-acid shows that absorption at this time is less evident than in untreated mice. Subsequently Rake[3] demonstrated that absorption through the nasal mucosa, chiefly the olfactory, is very rapid and has reached its maximum in mice killed at 2 minutes; furthermore, that much of the absorption occurs by way of the olfactory sensory cells. It seemed advisable to study the absorption of Prussian blue from the nasal cavities of tannic-acid treated mice during these early periods.

Nine Swiss mice each received eight preliminary intranasal instillations over 3 days. Each dose, consisting of 0.03 cc. of 0.8 percent tannic-acid in 1% glycerine, was dropped on the outside of the nostrils from whence it was gradually breathed into the nose. Four hours after the last dose each mouse received 0.02 cc. of a mixture of equal parts of 10% iron ammonium citrate and 10% potassium ferrocyanide intranasally by the same technique. Two minutes after commencement of intranasal inoculation 5 mice were sacrificed by decapitation and 13 minutes later the other 4 were so sacrificed. Fixation, sectioning, and staining with Mayer's carmine were carried out as indicated elsewhere.[3] A few sections were stained with hematoxylin and eosin to facilitate study of the inflammation.

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[8] Zondek, B., *J. Am. Med. Assn.*, 1935, **104**, 637.

[1] Clark, W. E. le G., *Great Britain Rep. Pub. Health and Med. Subj.*, Ministry of Health, 1929, No. 54.

[2] Olitsky, P. K., and Cox, H. R., *Science*, 1934, **80**, 566.

[3] Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 369.

Following intranasal treatment with tannic-acid as given above, it is very rarely that any mouse shows exudate from the nostrils. Nevertheless, it was found in the present study that there is to be observed, microscopically, marked exudate into the nasal cavity, especially over the olfactory mucosa and around the turbinates. This exudate is composed of mucus, leucocytes—chiefly polymorphonuclears—and some red cells. The olfactory mucosa and submucosa show swelling and infiltration with oedema fluid and leucocytes. The capillaries of the submucosa are dilated and contain many polymorphonuclear leucocytes. Changes in the respiratory mucosa are slight. Six mice show intense inflammation. In 3 the inflammation is less. Preliminary treatment with 1% tannic acid in normal saline, instead of 0.8% in 1% glycerine, is found to produce similar inflammatory changes.

At first sight the absorption of Prussian blue in these mice sacrificed 2 minutes after instillation does not appear to differ markedly from the absorption seen in untreated mice.[3] The granules have penetrated the mucosa, lie in the tissues and within lymphatics and capillaries, and are also found in the perineural spaces of the olfactory nerves, within the subarachnoid space and inside cells of the pia mater over the olfactory bulb. It is true that there is quantitatively less pigment in the perineural spaces or within the cranial cavity in these treated mice as compared to the normal, and that nerves so full of the granules as to appear as blue bands are very unusual, but the difference is one only of degree. However, when attention is turned to the method by which the pigment is passing through the olfactory mucosa a qualitative difference is found.

In 3 of the 5 mice examined at 2 minutes, careful search reveals Prussian blue inside at most 2 or 3 olfactory sensory cells. In the other two mice one can find a few areas in which as many as 8 or 10 olfactory sensory cells may contain pigment. The greater part of the absorption in all of these treated mice does not involve the olfactory sensory cells, as it does in the untreated group. It occurs between the cells of both olfactory and respiratory mucosa.

In the 4 mice examined 15 minutes after instillation of pigment the picture again differs only slightly from that seen in the untreated group. Only in the olfactory mucosa itself is there a difference and here again it is found that three of the 4 mice show no granules in the olfactory cells and one mouse shows considerably less than do the normal mice.

A correlation appears between the degree of inflammation of the mucosa and the passage of the Prussian blue into the olfactory cells. In those 6 mice in which pigment is seen in none or very few of these cells, the inflammation is intense and the exudate heavy. In the other 3 mice the inflammation is comparatively less and there is some, albeit slight, passage of granules into the olfactory cells. It appears that both departures from normal resulting from the tannic-acid treatment run parallel in the mouse. We have not investigated whether the inflammation is causally related to the decreased passage of pigment into the olfactory sensory cells.

8766 P

Effect of Progestin upon the Mammary Glands of the Mouse.*

W. U. GARDNER AND R. T. HILL. (Introduced by Edgar Allen.)
From the Department of Anatomy, Yale University School of Medicine.

The growth of the mammary glands of males bearing ovarian grafts or receiving extracts of estrogenic hormones has been observed by many investigators. Several pure estrogenic chemicals, theelin, theelol,[1] dihydroestrin and equilin,[2,3] induced the growth of the mammary glands of male mice and of males of other species. The male hormone, testosterone, also induced a limited mammary gland proliferation in rats.[4]

Progestin had no effect upon the mammary glands of rabbits when injected as a rather crude extract,[5,6] but when progestin and theelin were administered simultaneously a complete development of the mammary glands occurred.[7] The mammary ducts developed in rabbits receiving estrin, while simultaneous injections of progestin and estrin induced the development of the lobules of alveolar tissue as observed during pregnancy or pseudo-pregnancy. In some strains of mice scattered lobules of alveoli developed in males receiving large amounts of estrogenic hormone[8] while a complete development of the lobules occurred in injected males from other strains.[9]

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* This investigation has been supported in part by the Fluid Research Funds of Yale University School of Medicine and the Committee for Research in Problems of Sex of the National Research Council through grants administered by Professor Edgar Allen.

[1] Turner, C. W., *et al.*, *Anat. Rec.*, 1932, **63**, 227.

[2] Burrows, H., *Brit. J. Surg.*, 1935, **23**, 191.

[3] Gardner, W. U., *et al.*, unpublished.

[4] Selye, S., McEuen, C. S., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 201.

The mice used in this investigation were supplied by Dr. L. C. Strong. Two highly purified extracts of progestin obtained from corpora lutea† ‡ and one crystalline preparation of progesterone (Proluton) § were used. All of the preparations were dissolved in oil and were injected subcutaneously.

Seven castrated male mice were given from 0.35 to 1.6 units of progestin† over periods of from 14 to 16 days. Two of these mice were given in addition 5 rat units of theelin daily. The mammary rudiments of all of these mice had developed into extensive systems of branching ducts, similar to those of mice receiving theelin for approximately 30 days. One castrated male receiving 5 r.u. of theelin daily showed but a slight growth of the mammary glands. One of two non-castrate male mice receiving 0.1 unit of progestin daily for 14 days showed extensive development of the mammary ducts.

Three virgin female mice were given 0.15 unit‡ of progestin weekly in 3 injections. After periods of treatment ranging from 22 to 70 days the mammary glands consisted of branching ducts as in normal virgin females. Two normal male mice receiving similar treatment for periods of 22 and 39 days, and a total of 0.5 and 0.8 units showed a definite growth of the mammary rudiments. Six male mice were given 7.5 r.u. of theelin weekly for 3 weeks, and

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[5] Corner, G. W., *Am. J. Physiol.*, 1930, **95**, 43.

[6] Turner, C. W., and Frank, A. H., *Science*, 1931, **73**, 295.

[7] Turner, C. W., and Frank, A. H., *Mo. Agr. Exp. Sta. Res. Bull.*, 1932, 174.

[8] Gardner, W. U., Smith, G. M., and Strong, L. C., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 148.

[9] Unpublished observations of the authors.

† Progestin, containing 1 Corner-Allen unit per cc., was supplied by Upjohn Co. through the courtesy of Dr. G. F. Cartland.

‡ The progestin and theelin in aqueous solution were supplied through the courtesy of Dr. O. Kamm of Parke-Davis Co. The progestin contained 1 rabbit unit (Clauberg) per cc.

§ The Proluton ("pure crystalline corpus luteum hormone") (Progesterone) was supplied by the Schering Corp. through the courtesy of Dr. Gregory Stragnell. The solution used contained 1 i.u. per cc. (1 mg.).

then supplemented by 0.15 unit of progestin weekly in 3 injections for periods up to 110 days. Total doses of from 1.75 to 2.4 units of progestin were given. The mammary rudiments of all the mice had developed into moderately extensive duct systems.

Fourteen male mice were given Proluton§ in daily doses of 0.05 to 0.1 i.u. for periods of from 15 to 26 days. A definite growth of the mammary ducts was observed in 8 non-castrate and in 1 castrate animal. The rudimentary mammary glands of three mature mice receiving 0.05 i.u. and of 2 receiving 0.1 i.u. had failed to grow. Two non-castrate males and one castrated male were given 5 r.u. of theelin and 0.1 i.u. of Proluton daily. All of these animals showed definite growth of the mammary gland.

Two of the above preparations were assayed for the presence of estrogenic hormone. Two-tenths of a unit failed to give vaginal cornification or mucification in castrated female mice. The minimum amount of estrogenic hormone in oil solution which will give mammary growth has not been determined, but 1 r.u. of theelin in aqueous solution daily, when given in sufficient dilution in two injections, is sufficient. It has also been indicated that old male mice require larger amounts of estrogenic hormone to induce the growth of the mammary glands than younger mice. This might account for the failure of response in 5 of the animals receiving Proluton.

8767 C

Does Magnesium Sulphate Cause Catharsis by Being Absorbed and then Excreted into the Colon?

JOHN FERGUSON AND A. C. IVY.

From the Departments of Physiology and Pharmacology, Northwestern University Medical School, Chicago, and from the University of Alberta, Alberta, Canada.

There are numerous reports in the literature dealing with the mode of action of the saline purgatives. Invariably, 2 main theories are advanced. The first, favored by Meltzer and Auer,[1] Auer,[2, 3] Frankl,[4] Mendel and Benedict,[5] and Cohen,[6] suggests that the action is from the lumen of the intestine. The saline purgative when administered by mouth increases the fluidity and bulk of the feces by causing retention of liquid with some extraction of fluid from the blood; thus catharsis is produced. The salt which is absorbed is incidental and is excreted largely by the kidney, except in cases of nephritis in which the magnesium content of the blood may be increased to dangerous levels.[7, 8] The second theory, on the contrary, which is supported by the work of MacCallum,[9] Bancroft,[10] Hertz, Cook and Schlesinger,[11] and Robin and Sourdel[12] intimates that the saline purgatives act only after being absorbed and not from the lumen of the intestine. This theory is well stated by MacCallum,[9] who investigated the problem on the suggestion of Loeb, that, "the presence of the salt in the lumen of the intestine is not necessary for its cathartic action—the salt must be absorbed into the blood before it can act on the intestine."

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[1] Meltzer, S. J., and Auer, J., *Am. J. Physiol.*, 1905, **14**, 366.

[2] Auer, J., *Am. J. Physiol.*, 1906-7, **17**, 15.

[3] Auer, J., *J. Biol. Chem.*, 1908, **4**, 197.

[4] Frankl, T., *Arch. f. exper. Path. u. Pharmakol.*, 1907, **57**, 386.

[5] Mendel, L. B., and Benedict, S. R., *Am. J. Physiol.*, 1908-09, **23** (Proc. 18).

[6] Cohen, H., *Quart. J. Med.*, 1925-26, **19**, 249.

To determine whether magnesium sulphate was absorbed and excreted in quantity into the colon, we anesthetized several dogs (13 in all) using barbital, nembutal, or morphine and ether. The small intestine was isolated by tying it off at the stomach and the ileocecal valve; the large intestine was washed free from magnesium with warm isotonic sodium chloride solution—care being taken not to damage the intestinal mucosa. A solution of magnesium sulphate (10-15 gm. of the salt, according to the weight of the dog) at approximately 37.6°C. was injected into the small intestine, the abdomen closed and the temperature of the animal kept as nearly normal as possible. At the conclusion of the experiment, which lasted from ½ to 9 hours, the colon was washed out with distilled water and the magnesium of the washings determined following the method of McCrudden.[13, 14] The amount of magnesium excreted into the colon was found to be insignificant—invariably, less than ½ of 1% of the amount of magnesium injected into the intestine.

An ileostomy was performed aseptically on each of 3 dogs and on the third day after the operation the isolated colon and rectum were washed out with warm isotonic sodium chloride solution. The

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[7] Hirschfelder, A. D., *J. Am. Med. Assn.*, 1934, **102**, 1138.

[8] Hirschfelder, A. D., *J. Biol. Chem.*, 1934, **104**, 647.

[9] MacCallum, J. B., *Am. J. Physiol.*, 1903-04, **10**, 101.

[10] Bancroft, F. W., *J. Biol. Chem.*, 1907, **3**, 191.

[11] Hertz, A. F., Cook, F., and Schlesinger, E. G., *Proc. Roy. Soc. Med. Lond.*, 1908-9, **2**, Part 3 (Therap. and Pharmacol. Sect.), 21.

[12] Robin, A., and Sourdel, M., *Bull. d. l. Soc. Med. d. Hopt. d. Paris*, 1912, **38**, 881.

[13] McCrudden, F. H., *J. Biol. Chem.*, 1909-10, **7**, 83.

[14] McCrudden, F. H., *J. Biol. Chem.*, 1911-12, **10**, 187.

magnesium in the washings was determined. Magnesium sulphate was then given by stomach tube and the colon washed out at the end of 24 hours; the dog having not evacuated within that period. The magnesium was determined from the washings. In all, 5 sets of determinations were made before and after the administration of magnesium sulphate but the salt did not appear to be excreted into the colon to any extent.

Seven normal dogs were kept in separate stalls on a uniform diet. During the first period of 6 days the feces were discarded; during the second period of 6 days an attempt was made to obtain a normal pattern for a particular dog by collecting the feces as soon as possible after they had been excreted and noting the form, markings and other criteria of laxation. The water content was determined later. The feces of a particular dog appeared to be fairly uniform during this period. Then, magnesium sulphate was injected subcutaneously in some cases and intramuscularly or intravenously in others. In the event of a second injection several days were allowed to elapse between the injections. The injection of magnesium sulphate did not appear to influence significantly the water content, form, time, weight or other criteria of laxation as outlined by Cowgill and his coworkers.[15, 16] In some instances at a later date the dogs were given by stomach tube the same quantity of magnesium sulphate which they had been given intramuscularly and within 24 hours the stools indicated a laxative effect of the salt.

A dog was kept on a uniform diet for 17 days. During this period the dog was injected subcutaneously with 25% $MgSO_4.7\ H_2O$, 2 cc. per kg. bodyweight, and 2 days later was given a dose of magnesium sulphate by stomach tube. The comparative osmotic pressure which the dried material of each stool would exert was determined by the freezing point method. There was no significant change after the injection of the salt but after the administration by stomach tube there was evidence of increased osmotic pressure.

Conclusion. (1) Magnesium sulphate in the dog does not cause laxation or catharsis by being absorbed into the blood and then excreted into the colon. (2) The parenteral administration of magnesium sulphate does not augment the excretion of magnesium by the colonic mucosa to an appreciable extent.*

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[15] Cowgill, G. R., and Anderson, W. E., *J. Am. Med. Assn.*, 1932, **98**, 1866.

[16] Cowgill, G. R., Anderson, W. E., and Sullivan, A. J., *J. Am. Med. Assn.*, 1933, **101**, 273.

* This work was aided in part by a grant from the Carnegie Corporation of New York.

8768 C

Inhibition of Menstruation by Crystalline Progesterone.

George W. Corner and Willard M. Allen.

From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

It has been shown[1] that partially purified extracts of the corpus luteum, containing progesterone, possess the property of inhibiting menstruation in the monkey. In view of the possibility that contaminating substances were involved in this result, the experiments have been repeated on five animals with crystalline progesterone, both natural and synthetic.

The hormones used consisted of 2 lots of β-progesterone prepared in our laboratory from sows' ovaries, 2 lots of synthetic β-progesterone donated by the Schering Corporation through the kindness of Dr. Erwin Schwenk, and a lot of synthetic α-progesterone donated by E. Merck, Darmstadt. The animals were chosen and the experiments conducted in the same way as those of Corner, 1935.[1] Each animal was allowed one or more control cycles and the time of the next menses having been estimated as closely as possible from the history, treatment with progesterone was begun about a week before the expected onset. The dosage was uniformly 1 mg. daily, equivalent to 1 international unit. One animal received natural β-progesterone throughout. Two received the first of the Schering preparations for 11 days, and then the second Schering preparation for 5 and 6 days respectively; menstruation then being overdue, the experiments were continued with natural progesterone for a few days longer. Finally, 2 received the Merck α-progesterone throughout. In all 5 experiments, the expected menstruation was delayed for about 2 weeks, after which the hormone was discontinued according to plan. Bleeding began 2 to 4 days thereafter.

The only feature in which the result with crystalline progesterone differed from that with the partially purified substance used in previous experiments, was in the more rapid onset of bleeding after discontinuance, namely 2 to 4 days instead of 5 to 8. This may have been due to more rapid utilization or excretion of the pure hormone.

In conclusion, it may be stated that all 4 of the definitely established effects of corpus luteum extracts upon the uterus, namely progestational proliferation of the endometrium,[2] inhibition of action of pituitrin upon the myometrium,[3] inhibition of uterine motility *in vivo*,[4] and suppression of menstruation,[1] have now been achieved with crystalline progesterone.

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[1] Corner, G. W., *Am. J. Physiol.*, 1935, **113**, 238.

[2] Wintersteiner, O., and Allen, W. M., *J. Biol. Chem.*, 1934, **107**, 321.

8769 P

Effect of Thyroid Feeding and Thyroidectomy on Thymus Size in the New-born Rat.

D. BAILEY CALVIN. (Introduced by M. M. Ellis.)

From the Department of Biochemistry, University of Missouri School of Medicine, Columbia, Mo.

Certain clinical indications have led to the feeling that a possible interrelationship between thyroid size or condition in the mother and the size of the thymus in the offspring might be demonstrable. This has been investigated experimentally, using the albino rat as the experimental animal.

A study of basal metabolic conditions in normal and thyroid-treated animals has been made. Although somewhat inconclusive, due to variability greater than 10%, the results indicate that one milligram of desiccated thyroid (Parke-Davis) given orally per day will maintain the thyroidectomized rat of about 150 gm. weight in fairly normal metabolic balance.

With this determined, three experiments were set up, each group containing eight proven breeding females, whose normal litters had been used to establish normal thymus size. Group 1 received 2 mg. of desiccated thyroid per day; Group 2, 5 mg.; the females in Group 3 were thyroidectomized. Weight curves were kept on all animals for 2 weeks prior to pregnancy. Vaginal smears were done to determine the stages of estrus and the time of breeding to within 4 hours. All animals were fed on a diet containing ⅓ dried whole milk and ⅔ ground whole wheat, plus 1% sodium chloride and 1% calcium lactate. Lettuce and liver were given 3 times a week.

The results were as follows: Prior to pregnancy, the animals in Group 1 made a steady gain in weight; Group 2 gained, but somewhat more slowly; Group 3 showed some gain in weight. Repeated estrous cycles were seemingly normal for the thyroid-fed normal animals. This did not hold true, however, for the thyroidectomized females, where fairly normal cycles were obtained for 4 to 8 days after the operation, but after this, with 2 exceptions where breeding occurred, the animals went into diestrus and have remained in this stage for over 4 weeks.

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[3] Makepeace, A. W., Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1936, **115**, 376.

[4] Allen, W. M., and Reynolds, S. R. M., *Am. J. Obstet. and Gyn.*, 1935, **30**, 309.

The results on thymic size in the offspring are given in Table I.

TABLE I.

		Aver. thymus wt., mg.	Aver. thymus wt. per 5 gm. of new-born rat, mg.	Aver. wt. new-born rat, gm.
Normal.	Aver.	7.4	6.5	5.7
	Max.	7.8		
	Min.	7.0		
2 mg. thyroid per day	Aver.	5.7	5.3	5.4
	Max.	6.0		
	Min.	4.9		
5 mg. thyroid per day	Aver.	4.4	4.1	5.35
	Max.	5.0		
	Min.	3.4		
Thyroidectomy (only 2 litters)	Aver.	8.1	7.2	5.6
	Max.	8.3		
	Min.	7.9		

These results seem to indicate that excess of thyroid during gestation results in smaller thymus gland growth in the fetus, while low thyroid results in larger thymus glands.

Further experiments indicate that thyroid-fed mothers, although having given normal litters to weaning before thyroid feeding was instituted, are unable to nurse their offspring while receiving the thyroid. In the 2 cases observed, the thyroidectomized mothers seemed to have considerable difficulty at parturition.

An effort is being made to determine whether these results may be of significance in the clinical syndrome known as status thymicolymphaticus.

8770 P

Comparison of Precipitating Action of Basic Dyes on Bacteriophage and Bacterial Proteolytic Enzymes.

ARTHUR W. WALKER.

From the Department of Bacteriology, Northwestern University Medical School, Chicago, Illinois.

Safranin is able to remove a number of biological substances from solution. Robertson[1] observed that safranin when added to a solution of trypsin would cause a precipitate. Holzberd[2] showed that this precipitate was proteolytic and later Marston[3] proved that not only safranin but all the azin dyes removed trypsin and pepsin from solution. The azin dyes also quantitatively precipitate bacterial proteolytic enzymes.[4] Vinson[5] reported that the virus of the mosaic disease of tobacco was completely removed from the infected plant juice by safranin. Krueger and Baldwin,[6] in their studies on the inactivation of bacteriophage, used safranin as one of the inactivating agents, and observed a precipitate which partly but not completely removed the phage from solution.

We examined the action of the azin and other basic dyes on bacteriophage and compared the results with those obtained by the use of the same dyes with bacterial proteolytic enzymes.

The following chemical types of basic dyes were used: azin, thiazin, oxazin, xanthane and phenylmethane. The bacteriophage employed was a *coli*-phage made in synthetic medium and free from all protein except bacterial. The titer of the phage was $1x10^{-9}$. One cc. of the phage and 7 cc. of physiologic salt-solution were placed in a 15 cc. tube and 2 cc. of M/70 solution of the dye in water added. The mixture was allowed to stand for 18 hours at room temperature, and then centrifuged. The supernatant fluid was poured off and filtered through a Berkefeld N filter. This was found to be necessary as the unfiltered supernatant fluid of both the phage and the enzyme showed the presence of small amounts of lytic and proteolytic substance. The precipitate was taken up in 10 cc. of a pH 6.8 phosphate buffer solution.

Both the precipitate and filtrate were diluted in plain broth and

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[1] Robertson, T. B., *J. Biol. Chem.*, 1907, **2**, 343.

[2] Holzberd, H. L., *J. Biol. Chem.*, 1913, **14**, 335.

[3] Marston, H. R., *Biochem. J.*, 1923, **17**, 651.

[4] Walker, A. W., PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 839.

[5] Vinson, C. G., *Science*, 1927, **44**, 357.

[6] Krueger, A. P., and Baldwin, D. M., *J. Infect. Dis.*, 1935, **57**, 207.

TABLE I.

	Azin			Thiazin			Oxazin			Xanthane	Phenyl Methane
	Safranin	Neutral red	Janus green	Thionin	Methylene green	Toluidin blue	Brilliant cresyl blue	Cresyl violet	Gallocyanin	Pyronin	Malachite green
Bacteriophage											
Precipitate	+	+	+	+	+	+	+	+	+	+	+
Filtrate	−	−	−	−	−	−	+	+	+	+	+
Proteolytic enzyme											
Precipitate	+	+	+	+	+	+	+	+	+	+	+
Filtrate	−	−	−	−	−	−	+	+	+	+	+

Presence of phage or enzyme = +
Absence of phage or enzyme = −

tested for phage in the usual manner. For the bacterial proteolytic enzymes, a Berkefeld filtrate of a 4-day culture of *B. pyocyaneus* in synthetic medium was used in the same manner as the bacteriophage. The test for the presence of the proteolytic enzyme was by inoculating the filtrate and suspended precipitate into carbol-gelatin, incubating at 37°C. for 48 hours, placing in the icebox for 1 hour, and then observing for liquefaction.

Table I shows that the bacteriophage and the proteolytic enzyme are completely precipitated by these azin and thiazin dyes, and that the other basic dyes used do not completely remove the phage or the enzyme from solution. With the exception of pyronin the dyes had little if any inhibiting action on either the phage or the enzyme. In the case of the pyronin the inhibiting action was considerable for both.

This inhibitory action has not been investigated. It may be due to the photodynamic action of the pyronin. But some of the other dyes are photodynamically active and showed no such effects; further, *coli*-phage, unlike the staphylococcal phage, is not sensitive to oxidation.

The nature of the union of phage with the dye is unknown. If it is an adsorptive phenomenon one could expect that all the dyes of the same charge and relative molecular size would act in the same manner.

Marston believes the union of trypsin and pepsin with the azin dyes to be a chemical reaction.

The chemical union of bacteriophage and dye, if proven, would be another indication of the chemical or nonliving nature of the bacteriophage.

8771 C

Preganglionic Connections of the Intramural Ganglia of the Urinary Bladder.

RUSSELL L. MOSELEY. (Introduced by Albert Kuntz.)
From the St. Louis University School of Medicine.

The pelvic autonomic ganglia including the intramural ganglia of the urinary bladder, are connected with the lower thoracic and upper lumbar segments of the spinal cord through the splanchnic and hypogastric nerves and with the sacral segments through the pelvic nerves. The efferent fibers which enter the pelvic plexus on either side via the hypogastric nerve are mainly postganglionic sympathetic fibers, but include some preganglionic fibers of the thoraco-lumbar outflow. According to Langley and Anderson (1894, 1896)[1] and Stewart (1899)[2] some preganglionic fibers which enter the inferior mesenteric plexus do not terminate in this plexus but extend distalward in the hypogastric nerves. In spite of these reported observations, the pelvic autonomic ganglia quite generally have been regarded as related mainly to the parasympathetic division of the autonomic nervous system.

In an experimental anatomical study of the preganglionic connections of the ganglia in the pelvic plexus in the cat, Kuntz and Moseley (1936)[3] have demonstrated that some of these ganglia receive preganglionic fibers exclusively through the thoraco-lumbar outflow, some exclusively through the sacral outflow, and some through both the thoraco-lumbar and sacral outflows.

In the present study, cats have been used as the experimental animals, and the following operative procedures have been carried out under aseptic conditions. In one series, the pelvic nerve was sectioned bilaterally near its origin from the sacral nerves in order to insure degeneration of all preganglionic and visceral afferent sacral nerve components which enter the urinary bladder. In another series, the inferior mesenteric ganglia and both sympathetic trunks from the second lumbar segment caudalward were extirpated in order to insure degeneration of all preganglionic components of the thoraco-lumbar outflow which might reach the urinary bladder. When the animals were killed 18 to 24 days after operation, the urinary bladder was prepared for study by the pyridine silver method.

Preparations of the bladder taken from animals which have been subjected to section of the pelvic nerves show fiber degeneration in some of the intramural ganglia but not in others. In some ganglia, all the fibers except those of local origin have undergone degeneration; the intercellular plexuses formed by the preganglionic fibers which terminate in relation to the ganglion cells are absent. In some, the intercellular plexuses are absent in certain areas but present in others. In some, no evidence of fiber degeneration can be detected.

Preparations of the bladder taken from animals which had been

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[1] Langley, J. N., and Anderson, H. K., *J. Physiol.*, 1894, **17**, 177; *ibid.*, 1895, **20**, 372.

[2] Stewart, C. C., *Am. J. Physiol.*, 1899, **2**, 182.

[3] Kuntz, A., and Moseley, R. L., *J. Comp. Neur.*, 1936, **64**, No. 1.

subjected to extirpation of the inferior mesenteric ganglia and both sympathetic trunks from the second lumbar segment caudalward also show fiber degeneration in some of the ganglia but not in others. In some ganglia, all the fibers except those of local origin have undergone degeneration. In some, the intercellular plexuses have undergone degeneration in some areas but not in others. In still others, no evidence of nerve fiber degeneration can be detected.

The ganglia in which intercellular plexuses undergo degeneration following extirpation of the inferior mesenteric ganglia and the sympathetic trunks from the second lumbar segment caudalward obviously receive preganglionic fibers via the thoraco-lumbar outflow. Those in which intercellular plexuses undergo degeneration following section of the pelvic nerves obviously receive preganglionic fibers via the sacral outflow. Those which show degeneration of the intercellular plexuses in only a portion of the ganglion, after either type of operation, receive preganglionic fibers from both the thoraco-lumbar and sacral outflows. The findings set forth above, therefore, warrant the conclusion that some of the intramural ganglia of the urinary bladder receive preganglionic fibers exclusively via the thoraco-lumbar outflow; some exclusively via the sacral outflow, and some via both; consequently, they include sympathetic, parasympathetic and mixed ganglia.

Quantitative determinations of the sizes and distribution of the ganglia of these three types have shown that approximately 40% are parasympathetic, 40% sympathetic and 20% mixed. The parasympathetic ganglia on the average are larger than the sympathetic ganglia and are situated more superficially in the bladder wall, being located mainly in the adventitia and the outer zone of the musculature. The ganglia located more deeply in the musculature are predominantly sympathetic. Although the ganglia are most abundant in the trigone, particularly in proximity to the ureters, those within this area make up only 50% of the intramural ganglia and include parasympathetic and sympathetic ganglia in approximately equal numbers. These findings do not substantiate the conclusions of Iljina and Lawrentjew (1932)[4] and Polykarpowa (1935)[5] that the intramural ganglia of the urinary bladder are exclusively parasympathetic.

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[4] Iljina, W. S., and Lawrentjew, B. J., *Ztschr. f. mikr. Anat. Forsch.*, 1932, **30**, 543.

[5] Polykarpowa, A., *Ztschr. f. Anat. u. Entwg.*, 1935, **104**, 378.

8772 P

Micro-Determination of Fluorine.

WALLACE D. ARMSTRONG. (Introduced by F. H. Scott.)
From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.

The author's[1] modification of the Willard-Winter[2] method has been adapted to the determination of small quantities of fluorine. The distillation of H_2SiF_6 is carried out in the usual manner except that a 50 cc. distilling flask, 0.1 gm. quartz and 5 cc. 60% perchloric acid are employed. The temperature of the flask contents is maintained at 140°, and 150 cc. of distillate are collected. The water employed is freshly boiled after redistillation from alkali. The distillate is made alkaline to phenolphthalein, evaporated to a small volume, rendered just acid to the indicator, and diluted to 10 cc. One cc. aliquots are transferred to 5 cc. vials (such as those in which dentists receive gold foil). The addition of an equal volume of alcohol is omitted in order to permit a direct relationship between the fluorine content of the aliquots and the volume of $Th(NO_3)_4$ solution used for their titration and to render inoperative the effect of $HClO_4$ on the determination of more than 5γ (micrograms) F. If the sample contains less than 5γ F, 6 gm. $NaClO_4$ are added to the charge in the distilling flask, the distillate evaporated to about 5 cc. and the aliquots taken by weight. $NaClO_4$ reduced the amount of $HClO_4$ volatilized during the distillation of H_2SiF_6. One drop of 0.05% sodium alizarin sulphonate is added to the aliquots and, after split drops of appx. 0.03 *N* HCl are added until the indicator has a light orange color, one drop of buffer soln. (1 molar monochloracetic acid and 1 molar sodium monochloracetate) is introduced. The fluorine is titrated with appx. 0.0004 *N* $Th(NO_3)_4$ (1 cc. $= 7\text{-}8\gamma$ F) to the appearance of a barely perceptible pink color which matches that of a titrated standard solution containing 5γ F. The $Th(NO_3)_4$ solution is standardized daily by titration of 1 cc. volumes of solutions containing 10, 5 and 1γ F. The results are substituted in the two-point equation for a straight line and the titration blank calculated.

Five analyses each of 25 and 10γ quantities of F gave an average recovery which was 1.4% low. Seven distillation blank determinations in which 25 and 10γ of fluorine were added to the receiver contents gave an average recovery 2.5% low. The quantity of fluorine in each aliquot titrated was one-tenth the total in distillate. Four analyses each of 5 and 2γ quantities gave results 1.5% low while corresponding distillation blank determinations averaged 0.5% low.

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[1] Armstrong, W. D., *J. Am. Chem. Soc.*, 1933, **55**, 1741.

[2] Willard, H. H., and Winter, O. B., *Ind. Eng. Chem., Anal. Ed.*, 1933, **5**, 7.

Four analyses of a quantity of mixed sound human enamel gave the results 0.0163, 0.0154, 0.0164, 0.0160% F. Three determinations were made in which 10 micrograms F were distilled from the residue in sample. The average recovery was 10.1γ F. Three analyses in which the distillate was redistilled and one in which it was twice redistilled showed 0.0158% F in the enamel. These latter results indicate that phosphate or any other substance in enamel is not volatilized in a sufficient quantity to affect the results.

The average recovery of 5γ F added to enamel was 4.8γ. Two determinations using $NaClO_4$ showed the enamel to contain 0.0160% F. A sample of C.P. "$Ca_3(PO_4)_2$" contained 0.622% F and one of C.P. $Ca(H_2PO_4)_2$ contained 0.013% F. The method has been successfully applied to the determination of F in dentin and will be extended to other biological material.

8773 C

Comparative Studies of Gonadotropic Hormones.* 5. Growth Response of Rat Mammary Glands in Chronic Experiments.

NELSON J. HOWARD. (Introduced by C. F. Fluhmann.)

From the Departments of Surgery and Obstetrics and Gynecology, Stanford University School of Medicine.

A description of the changes induced in the ovaries and hypophysis of female rats following the prolonged administration of gonadotropic extracts prepared from sheep anterior pituitary glands and blood of pregnant women was given in the fourth report of this series (Fluhmann[1]). The present communication deals with the gross and microscopic effects observed in the mammary glands of these rats.

Sheep Anterior Pituitary Extracts. Six rats received daily injections for 63 days. Breast biopsies showed proliferation of ducts with occasional branching tubules, but without colloid secretion or formation of alveolar structures. There was marked periductile fibrosis present in all animals. The injections were continued for 94 days, and 3 rats autopsied at that time were found to have the periductile fibrosis more marked than the previous series, while in 2 animals, fibroadenomatous proliferation of unencapsulated duct-containing fibrous tissue was present. In 5 rats injected for 149 days similar but more marked changes had taken place. After 179 days of injection, 5 rats showed only extension of periductile fibrosis and in 4 out of the 5 animals, fibroadenomatous areas were present as well. No cystic changes or alveolar development were noted in any of the above animals. This is in agreement with unpublished experiments carried out the previous year with acid sheep anterior pituitary extract. In previous experiments, 4 of 10 rats injected for 119 days showed, in addition to the periductile fibrosis and fibroadenomatous areas, large areas of microscopic and macroscopic cystic changes with colostrum-like secretion very similar to the cystic mastitis of women. Periductile fibrosis and fibroadenomatous areas were also found in rats given a rat anterior pituitary preparation daily for 105 and 165 days, and a human anterior pituitary extract prepared in the same manner injected for 119 days. The nipples of the rats injected with the pituitary extracts remained grey-white and non-erectile on stimulation, and showed no gross growth or elongation. No alveolar or lobule development was observed in any animals injected with acid anterior pituitary extracts. Normal virgin immature and mature female albino rats' breasts microscopically show only a fatty stroma about ducts and infrequent tubules, with only small amounts of fibrous tissues in the walls of the ducts. Consequently, periductile fibrosis and, now, non-encapsulated fibroadenomatous changes are distinct departures from the normal.

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*Supported in part by the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

[1] Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 691.

Pregnancy Blood Extract. The pregnancy blood extracts exerted a powerful stimulating effect on the unfolding of the alveolar mammary tree in virgin immature albino rats. Eleven rats receiving the extract for 28 days showed distinct development of alveoli and lobules, with many branching tubules and occasional areas of colloid secretion. After 59 days of such injections, 7 immature rats responded with marked lobular formation and clusters of alveoli, the lining cells of which were vacuolated and the lumen contained plentiful colloid secretion. By the end of 90 days 6 rats showed extensive lobule and alveolar development with marked vacuolation of alveolar lining cells, and plentiful colostrum and milk secretion filling alveoli and ducts. Five rats injected for 193 days had breasts in which microscopic sections revealed extensive lobules with both advanced lactation and regressive changes present. Many inspissated concretions were found in alveoli and ducts. One true encapsulated adenoma was present. After 272 days of injection, 4 rats showed marked regressive changes in the well developed alveoli and lobules, and although milk secretion was present it was limited to local areas of gland tissue. Two of the 4 animals had definitely encapsulated adenoma, while small microscopic milk cysts were present in all animals. Concretions were present in ducts and alveoli in each instance. The injections were continued in 2 animals for 365 days. One, No. 25, revealed marked regressive changes evidenced by shrunken, compact alveoli, concretions and pigment deposits. Animal No. 37 had marked hyperplasia of the alveolar lobules with extensive secretion changes, colloid and milk production. Small cystic areas were filled with colostrum and milk. In all animals receiving human pregnancy blood extract the nipple became pink, erectile, and elongated, and remained so throughout the experiment. The alveolar and lobule growth was not obtained in castrate 20-day and mature 114-day animals after 30 days of injection. The controls for animals receiving pregnancy blood extract had only small ducts and scanty tubules in the fatty breast tissue, with no evidence of alveolar growth.

Conclusions. The long-continued injection of acid anterior pituitary extracts and acid extracts of human pregnancy blood produced markedly different growth effects in the female albino rat breast. The acid pituitary extract response was characterized by growth and proliferation of the duct system with increasing periductile fibrosis and the appearance of fibroadenomatous areas, but without alveolar or lobule growth proliferation. In response to extracts of human pregnancy blood there was an unfolding, growth and hyperplasia of the alveolar mammary tree with secretion changes, milk production, and after long continued injections, regressive changes, often accompanied by the appearance of adenomatous tumors of the breast.

8774 P

Production of Fibrinolysin *In Vivo.*

E. Neter. (Introduced by E. Witebsky.)

From the Bacteriological and Serological Laboratories of the Beth Israel Hospital, New York City.

It was recently reported[1] that the fibrinolysin of hemolytic streptococci may be found *in vivo*. The fibrinolytic agent could be demonstrated in the spinal fluid of patients with purulent meningitis due to *streptococcus hemolyticus*. Subsequently, purulent exudates from various sources have been examined for fibrinolytic potency. The exudates were centrifuged immediately and the supernatant fluids were tested according to the technic of Tillett and Garner.[2]

Of 5 spinal fluids from patients with hemolytic streptococcal meningitis, 4 contained fibrinolysin; the fifth did not, in spite of the fact that the isolated strain produced fibrinolysin *in vitro*. Of 6 patients with pneumococcal meningitis, only one, due to pneumococcus type XIV, displayed fibrinolytic activity. The spinal fluids of 5 patients with meningococcal meningitis, and of a patient with tuberculous meningitis (proven bacteriologically), did not contain any fibrinolysin; nor did the spinal fluids of 4 patients with a clinical diagnosis of encephalitis.

Exudates from patients with purulent pericarditis, pleuritis and peritonitis also have been examined for fibrinolytic activity. The fibrinolysin was demonstrated in the peritoneal exudate of a patient with hemolytic streptococcal peritonitis and in the pleural fluid of another patient with β-streptococcal empyema. Fibrinolysin was also found in the exudates of 2 patients with pericarditis and peritonitis, respectively due to *Staphylococcus aureus hemolyticus*, the organism was present in the blood stream of both patients.

In order to determine whether fibrinolysin is produced in experimental animal diseases, various strains of pneumococci and hemolytic streptococci, suspended in saline, were intraäbdominally injected into white mice. The injected material was not fibrinolytic. Shortly after death of the mice, which usually occurred between 12 and 48 hours after the injection, the abdominal cavity was washed with about 10 cc. of normal saline; the specimens obtained were centrifuged and the supernatant fluid tested for fibrinolytic activity. Fibrinolysin was present in the washings from mice which had succumbed to hemolytic streptococcal infection. The fibrinolysin from washings dissolved clotted plasma; it was effective in high dilutions on human, but not rabbit or guinea pig plasmas; it was heat-labile, and could be neutralized by specific streptococcal antisera. The washings also contained hemolysin in high titer. Further experiments are necessary to determine whether the abdominal washings from mice infected with microörganisms other than hemolytic streptococci may contain fibrinolysin. Thus far, the washings from mice killed by various types of pneumococci did not display any fibrinolytic activity.

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[1] Neter, E., and Witebsky, E., *J. Bact.*, 1936, **31**, 77.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

8775 P

Toxic Effect of Various Concentrations of Bile on Dog's Gall Bladder.*

EDMUND ANDREWS AND HANS G. ARONSOHN.

From the Department of Surgery, The University of Chicago.

Experimental increase in gall bladder bile of one or several bile salts causes a reaction on the gall bladder wall. This increase can be produced in different ways: 1. By the addition of bile salts to whole gall bladder bile. 2. By concentrating the dog's own bile in the gall bladder. 3. By replacing the gall bladder bile with bile from another animal, concentrated previous to the introduction.

The first method has been applied in an experimental study,[1, 2] revealing the high toxicity of desoxycholic acid as compared with the weaker action of other bile salts.

Attempts have been made by us to concentrate gall bladder bile experimentally without removal from the gall bladder, thus producing changes in the gall bladder wall by means of the animal's own bile. These attempts, however, have met with great difficulties. Since all of the water-absorbing chemicals cause severe damage to the gall bladder wall, we tried to accomplish this concentration by washing the gall bladder bile with dry warm air for a certain length of time. This procedure resulted in some instances in an increase in the solid substances of bile, accompanied by a slight reaction on the wall of the gall bladder. However, the number of positive results is still too small to draw any conclusions.

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* Work done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Aronsohn, H. G. and Andrews, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 87.

[2] Andrews, E., and Aronsohn, H. G., *loc. cit.*

The third method mentioned, namely, the replacement of gall bladder bile by bile concentrated previous to the introduction, proved to be more satisfactory. In fact, the results are sufficiently convincing to be used for quantitative study.

The concentrating procedure was as follows: Dishes, each containing 200 cc. of fresh ox bile, were exposed to an electrical fan for different periods of time, allowing evaporation and concentration down to 150, 110, 88 and 60 cc. These different biles were injected by the catheter method into the dog's gall bladder. The results are given in Table I.

TABLE I.

	No. of Expts.	Results
Ox bile plain	3	Gall bladder practically normal in all instances.
" " conc. 150	5	Gall bladder normal in 2 cases, slight reaction in 3 cases.
" " " 110	6	Moderate degree of cholecystitis 2 cases, moderate degree of cholecystitis plus bile peritonitis 2 cases, severe cholecystitis 2 cases.
" " " 88	6	Severe cholecystitis 1 case, severe cholecystitis plus bile peritonitis 1 case, death and severe reaction 4 cases.
" " " 60	4	Severe cholecystitis 1 case, severe cholecystitis plus bile peritonitis 1 case, death plus severe reaction 2 cases.

Since bacterial culture revealed bacteria to be present in a great number in these concentrated biles, the following procedure was performed, to eliminate infection as the possible cause of the gall bladder reaction: The different bile concentrations were rediluted to the original volume (200 cc.) and this rediluted bile, serving as a control, was injected into the dog's gall bladder. No reaction was obtained, as seen from Table II, which proves that bacterial infection was not the cause of the changes in the gall bladder.

TABLE II.

	No. of Expts.	Results
Ox bile conc. 150, rediluted	2	Gall bladder normal
" " " 110, "	2	" " "
" " " 88, "	2	Normal 1 case, slight reaction 1 case.
" " " 60, "	2	Gall bladder normal.

Summary. 1. Concentration of ox bile more than half, has a marked toxic effect on the dog's gall bladder, causing death and bile peritonitis in many instances. 2. The foreign matter in bile is not the cause of these changes since the gall bladder is not affected by bile which is not concentrated. 3. Redilution of concentrated bile makes the bile ineffective, proving that infection is not responsible for the change.

8776 P

Sickle Cell Anemia In Deer.*

EARL C. O'ROKE. (Introduced by R. Isaacs.)

From the School of Forestry and Conservation, University of Michigan, Ann Arbor.

Although the presence of the "sickling" phenomenon has been described in man, presumably limited to the negro group, its presence in animals has been noted but rarely. Langeron[1] reported "demi-lune" forms of red blood cells of white rats and guinea pigs with rickets, and he reproduced these forms by the injection of lead acetate into healthy animals. It is not known, however, what relation these cells had to the phenomenon of sickling.

During the course of the study of 178 deer in the past 8 years, 14 were found in which the phenomenon of sickling was clearly demonstrated. One animal was found in Marin County, California, but all of the others were from 2 regions in the lower peninsula of Michigan, one near Alpena and the other near Bitely. All of the animals in these regions did not show sicklemia, however. The localization of the groups of affected animals suggests a hereditary possibility as is noted in human cases. In 100 autopsies on animals shot in the Edwin S. George Reserve of the University of Michigan, no animals showed sickling of the red blood cells. This herd is closely inbred.

This phenomenon was noted in animals shot for specimens, as well as in those found dead in the snow or otherwise well preserved. The cells in fixed films showed elongated, crescent shapes, with pointed ends, and relatively wider bodies (Fig. 1). The phenomenon was observed in fresh mounts sealed with petrolatum and allowed to stand. They resembled in every respect similar cells noted in human cases.

A rather striking similarity of symptomology was also noted. In those individuals in which field conditions permitted a thorough

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*Specimens for autopsy, courtesy Michigan Department of Conservation.

[1] Langeron, M., *Compt. rend. Soc. de biol.*, 1911, **70**, 434.

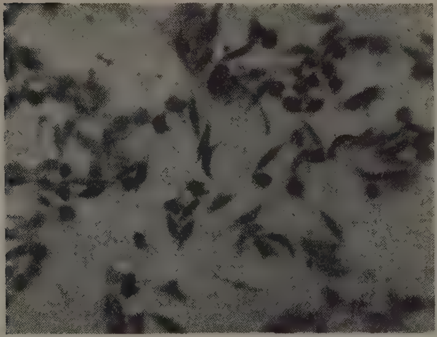

Fig. 1.
Blood film of white-tailed deer (heart blood) showing sickling phenomenon of red blood cells (Giemsa's stain).

autopsy, the animals were thin, emaciated and the blood appeared thin and watery. Atrophy of the spleen was noted in practically all of the affected deer. A similar condition has been noted in man.[2] Coincidental infestation with the deer louse fly, *Liptotena depressa*, or with lungworms was noted in some specimens. The abnormal frequency of broken leg bones in animals found dead in regions where sickling was observed suggests an analogy to possible bone changes (thinning of the cortex) noted in some human patients.[3]

It is possible that sickle cell anemia may be a factor, in addition to malnutrition, starvation and lungworm pneumonia, in the cause of the winter losses of deer in Michigan.

8777 C

Reaction of Some Rabbit Tissues to Graded Quantities of Trypan Blue.

Duncan C. Hetherington and R. L. McMillan. (Introduced by F. H. Swett.)

From the Department of Anatomy and the Department of Medicine, Duke University School of Medicine.

During the past 65 years a tremendous amount of effort has been directed toward the investigation of the nature and function of a large group of tissue cells designated collectively as the "reticulo-endothelial system". The principal tissues considered in these studies have been the liver, spleen, lymph nodes, bone marrow and the lining structures of the serous cavities.

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[2] Steinberg, B., *Arch. Path.*, 1930, **9**, 876.

[3] Grinnan, Andrew G., *Am. J. Roentg. and Rad. Therap.*, 1935, **34**, 297.

Ponfick[1] seems to have initiated the method of study most widely used; namely, the intravenous injection of particulate material. He employed cinnabar (red mercuric sulphide) and demonstrated its disposition in certain non-parenchymatous cells of the liver. It remained for von Kupffer,[2, 3] however, to identify these cells as phagocytes and to publish his classic descriptions. Since then many workers have contributed further to this study, notably Evans,[4] Evans and Scott,[5] Downey[6] and Evans and Schulemann.[7, 8, 9]

It is evident from all these investigations that a vast number of phagocytic cells, which accumulate and store particulate dyes to an extraordinary degree, exists within the animal body. Only after large quantities of dyes have been injected do fibroblasts and certain other tissue cells take up these substances in any great quantity.

It was thought that a study of the reactions of rabbit tissue to graded doses of trypan blue might show a differential storage of dye in certain types of tissue cells which do not belong to the histiocytic system.

Methods. Eight litter-mate Chinchilla rabbits in sound health were selected, housed and fed under constant conditions. They received by ear vein a sterile, 1% watery solution of trypan blue in 1.0 c.cm. quantities every 24 hours for the first 2 days. Thereafter the dosage was slowly increased until 5 c.cm. were given daily. The total dosages for rabbits numbered 1 to 8 were 5, 10, 20, 40, 50, 70, 85 and 100 c.cm.

The animals were killed and the tissues studied 24 hours after each had received its last injection of dye. Fresh tissue spreads were observed and material was also taken for fixation in 10% neutral formalin and in a modified Carnoy Lebrun's[10] solution. Sections were stained in carmalum.

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[1] Ponfick, Emil, *Arch. f. path. Anat.*, 1869, **48**, 1.

[2] von Kupffer, C., *Arch. f. mikr. Anat.*, 1876, **12**, 353.

[3] von Kupffer, C., *Arch. f. mikr. Anat.*, 1899, **54**, 254.

[4] Evans, Herbert M., *Am. J. Physiol.*, 1915, **37**, 243.

[5] Evans, Herbert M., and Scott, Katherine J., *Contrib. Embryol., Carnegie Inst.*, 1921, **10**, 3.

[6] Downey, Hal., *Anat. Rec.*, 1917, **12**, 429.

[7] Evans, Herbert M., and Schulemann, W., *Science, N. S.*, 1914, **39**, 443.

[8] Evans, Herbert M., and Schulemann, W., *Deutsche med. Wchnschr.*, 1914, **40**, 1508.

[9] Evans, Herbert M., and Schulemann, W., *Folia haemat.*, 1915, **19**, 207.

[10] Hetherington, Duncan C., and Tompkins, Edna H., *Stain Technology*, 1933, **8**, 31.

Kidney, liver, lung, adrenal and spinal cord were studied in particular for their reactions to the trypan blue. Other organs and tissues were also fixed, sectioned and studied largely as a check on the storage of dye in the non-parenchymatous cells.

Results. It was obvious from gross inspection of the animals that their tissues became progressively bluer with increasing doses of the dye. Sections of the tissues, as well as fresh spreads, showed that there was a differential storage of dye in certain cells other than those of the histiocytic system.

Dye became visible in the cells of the proximal convoluted tubules of the kidney after administration of only 5 c.cm. of trypan blue. The deposits were few and small and were found near the nucleus at the base of the cell. Progressively, as the dye was increased in quantity, the size and number of granular deposits increased in the tubular epithelium. In the animal receiving 100 c.cm. of trypan blue, not only was the dye present in greatest quantity in the cells of the proximal convoluted tubules but it was also visible in lesser amounts in the cells of the distal convoluted tubules and least of all in the loops of Henle. None could be detected in the epithelium of the collecting ducts. In the heavily dyed animals the epithelium of the proximal convoluted tubules was so crowded with dye accumulations that the outlines of the cells were obscured and in some cases the cells seemed to have become desquamated.

The livers of the lightly stained rabbits showed no evidence of storage except in the cells of von Kupffer. However, after 20 or more c.cm. of dye had been administered, there was a steady increase in accumulation of dye within the hepatic epithelium. At first the numbers of granules were few; later the dye was taken up in amazing quantities but not quite to such an extent as by the epithelium of the kidney. The Kupffer cells were not only enlarged remarkably within the sinusoids but they were increased in numbers and were so loaded with dye that it appeared to be in concentrated masses rather than in discrete granules. The mesothelial cells of the peritoneal surface of the heavily dyed livers also contained a few blue granules.

The lung tissues of the rabbits which had received small quantities of dye were free of colored pigment save that confined to the histiocytes which were not at all numerous. As the dosage of trypan blue was increased these cells became very noticeable not only by their numbers but also by the large quantities of dye stored in them. In some sections numerous dye filled histiocytes were found in the blood vessels, suggesting that many of them were carried to the lungs by way of blood coming from the liver.

The alveolar epithelium contained some blue granules in those animals which had received 40 or more c.cm. of dye. Only in the most heavily stained rabbits did the dye appear in the bronchiolar epithelium and then it did so to such a very slight extent that it was found only after careful search.

The epithelium of the adrenal cortex began to accumulate trypan blue in very small quantities only after 50 c.cm. of dye had been administered. In the highly stained rabbits blue granules, small in size and diffusely scattered, appeared particularly in the cells of the glomerular zone; the cells of the fascicular and reticular zones showed in comparison very few colored granules. Even fewer and finer dye accumulations could be seen in the cells of the medulla. Also very small, yet definite, amounts of dye were present in the endothelial cells of the medullary sinusoids.

In the spinal cord, dye was stored within the relatively rare histiocytes of the meninges but in no case could any trace of blue pigment be found in the neurons or glial cells. In spite of the large amounts of trypan blue in the other tissues of the heavily stained rabbits, the sections of spinal cord were singularly pallid.

Three of the rabbits studied were females. Sections of the ovaries showed that trypan blue was taken up in marked quantities by the low columnar cells composing the germinal epithelium. Other dye filled cells belonged to the macrophage group.

Conclusions. Following the administration of total graded doses of 5 to 100 c.cm. of 1% trypan blue to rabbits, the epithelium of the convoluted renal tubules, of the liver, of the pulmonary alveoli and of the adrenal cortex stored dye in decreasing amounts in the order named. The quantity of dye stored in a given tissue varied with the total quantity of dye administered.

In highly stained rabbits minute amounts of dye were observed also in the bronchiolar epithelium, in the endothelium of the medullary sinusoids of the adrenal and in the mesothelium of the liver, while larger amounts were present in the germinal epithelium of the ovary.

8778 C

Shock in Bile Peritonitis.*

VIRGIL H. MOON AND DAVID R. MORGAN.

From the Department of Pathology, Jefferson Medical College, Philadelphia.

The toxicity of bile and its salts has long been recognized. Either whole bile or the cholic salts produce severe systemic intoxication when injected, and produce intense local irritation of the tissues at the site of injection. (See Horrall[1] for review of literature.)

Horrall and Carlson[2] found that intraperitoneal injection of sterile bile produced severe illness characterized by vomiting, diarrhea, oliguria, albuminuria, bradycardia, low blood pressure and death in coma within 24 hours. They described edema, congestion and petechial hemorrhages of the lungs, brain, gastrointestinal mucosa, and marked congestion of the liver and kidneys as postmortem findings following bile peritonitis. These physiologic and pathologic features are characteristic of shock, whether occurring clinically or experimentally.[3] Similar disturbances followed intravenous or subcutaneous injections of bile. A small amount injected rapidly caused death promptly, while larger amounts given slowly caused severe illness followed by death from infection. Sublethal doses rendered dogs *especially susceptible to pneumonia.*

We have shown that edema of lungs is a characteristic feature of shock and predisposes to infection. Terminal pneumonia regularly developed following sublethal shock resulting from bile or from other agents.[4] This confirmed our observations[5] on terminal pneumonia following sublethal shock in man. The results of Horrall and Carlson were published before attention had been called to the pathology of shock[6] and to pneumonia as a sequella.

Shock has been studied by narcotizing an animal with barbital or similar drug, and recording blood pressure and pulse rate kymographically through an arterial cannula. Shock is then induced by severe tissue abuse such as trauma to the muscles. This method is open to serious objections. Variations in blood pressure and

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* Aided by the Martin Research Fund.

[1] Horrall, O. H., *Phys. Rev.*, 1931, **11**, 122.

[2] Horrall, O. H., and Carlson, A. J., *Am. J. Physiol.*, 1928, **85**, 591.

[3] Moon, V. H., *Ann. Int. Med.*, 1935, **8**, 1633.

[4] Moon, V. H., and Morgan, D. R., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 560; *Arch. Path.*, 1936, **21**, 565.

[5] Moon, V. H., *Am. J. Path.*, 1933, **9**, 899.

[6] Moon, V. H., and Kennedy, P. J., *Arch. Path.*, 1932, **14**, 360.

pulse rate often result from other conditions. Deep narcosis with barbiturates causes low blood pressure and other shock-like manifestations. Hemorrhage occurs incident to the trauma. Sublethal degrees of shock cannot be studied, since the animal cannot be kept in a condition suitable for subsequent observations. The recorded phenomena may be due to the narcotic, the trauma, the associated hemorrhage, or to a combination of these indeterminate factors.

These objections were obviated by producing shock without trauma and without narcosis, using variations in hemo-concentration as a physiologic criterion. Both lethal and sublethal degrees of shock can be produced and observations made on subsequent days. The accuracy of these was confirmed by the presence of the gross and microscopic features distinctive of shock.

Our results following injections of bile were like those of Horrall and Carlson except in one particular: their postmortem data included congestion of the spleen. We found the spleen firm, dry and relatively anemic following injections of bile and following shock otherwise produced.

A dog weighing 9 kg. was given intravenous injections of 7.5, 5.0, 7.5, 15 and 15 cc. of sterile normal dog bile at intervals of 2 hours. He became acutely weak and ill, showed muscular tremors, vomited, and the urine was scanty and deeply bile tinged. Twenty-four hours later the red cell count had risen from 6,100,000 to 8,000,000, indicating hemo-concentration of about 30%. The illness did not progress and the hemo-concentration three days later had declined to 15%. The dog was then killed and examined. The lungs were congested and somewhat edematous. There were petechiae in the pleura, and congestion of the liver and kidneys. There was no excess of fluid in the serous cavities. The spleen was firm, dry and anemic.

A dog weighing 9.3 kg. received intravenous injections of sterile dog bile, 40, 40, 50 and 60 cc. at intervals of 24 hours. Signs of illness and hemo-concentration were as described. He died 6 days following the first injection. The postmortem findings were as in the previous experiments except that the congestion and edema were more marked (Fig. B) and involved also the gastro-intestinal mucosa.

Sterile bile diluted with saline solution was injected into the peritoneal cavity, 5 cc. per kg. of weight. The dog promptly became ill as following intravenous injections of bile. In 3 hours the red cell count rose from 5,530,000 to 7,290,000; in 8 hours the count was 8,720,000, indicating hemo-concentration of about 57%.

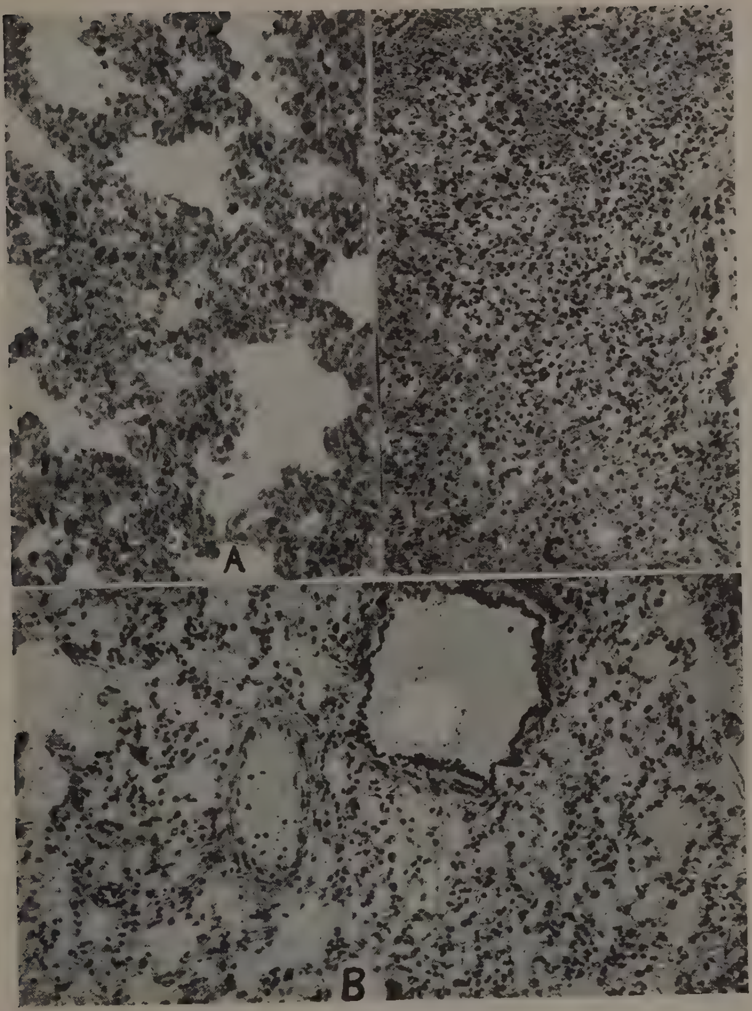

FIG. A.

Lung following death from bile peritonitis. Marked engorgement of the capillaries and slight edema are shown. x 1000.

FIG. B.

Pulmonary congestion and marked edema in shock with delayed death following repeated intravenous injections of bile. Such lungs are prepared for the development of pneumonia. x 200.

FIG. C.

Incipient pneumonia in congested edematous lung in sublethal shock. x 200.

He died the following night. The pleural, pericardial and peritoneal surfaces were deeply congested and contained blood-tinged fluid: 25 cc. in either pleural sac, 10 cc. in the pericardium and 100 cc. in the peritoneum. The lungs were heavy and intensely congested. The vessels along the mesenteric attachment were greatly engorged and prominent. The mucosae of the stomach and intestines were deeply congested and resembled purple velvet. There was marked congestion of the liver, pancreas and kidneys. The spleen was firm, contracted and dry. Microscopic examination showed marked capillary and venous engorgment, moderate edema and capillary hemorrhage in the lungs (Fig. A) and gastro-intestinal mucosa; marked congestion, parenchymatous degeneration and necrosis of the liver; marked congestion, capillary hemorrhages and parenchymatous degeneration of the kidneys, and moderate congestion of the pancreas and adrenals.

Two other dogs were similarly treated with exactly similar results. A fourth dog weighing 8.5 kg. received 25 cc. of bile intraperitoneally. This animal showed moderate illness and hemo-concentration, but he recovered.

Four dogs received 10% solution of sodium glycocholate intravenously. A dose of 1 gm. per kg. of body weight caused immediate death in one. Another received 8 doses of 0.4 gm. per kg. of body weight at daily intervals. This produced progressive illness and hemo-concentration, 35%, ending in death on the eighth day. Another received 10 injections of 0.4 gm. per kg. with intervals of 1 or 2 days, during 25 days. Moderate illness and hemo-concentration progressed to death 4 days following the last injection. The fourth dog died of acute shock 3½ hours following an intravenous injection of 0.4 gm. per kg. The postmortem findings in each case were those characteristic of shock. But following prolonged sublethal shock there was marked pulmonary edema and terminal pneumonia (Fig. C).

Recently Harkins *et al.*[7] made experiments on bile peritonitis with results similar to those described. In a series of 12 dogs the results were uniform and were recognized as shock. Hemo-concentration, low blood pressure, decreased blood chlorides and increased non-protein nitrogen were noted. They stated that the peritoneal cavities contained quantities of fluid resembling blood plasma, but did not state whether other congestive, edematous and hemorrhagic features were present. Such postmortem features are of especial significance when shock is under consideration. They interpreted bile peritonitis as causing death by shock, but emphasized the local loss of fluid as the shock-producing factor.

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[7] Harkins, H. H., Harmon, P. H., Hudson, J., and Andrews, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 691.

Our studies indicate that shock results from atony and dilatation of the capillaries and venules in visceral areas. Their permeability is increased, allowing leakage of plasma into the tissue spaces. This results in hemo-concentration and tissue edema, and leads to circulatory failure. The capillaries and venules are visibly engorged and packed with corpuscles. That the fluid in the tissue spaces and cavities results from leakage of plasma was shown by intravenous injections of trypan blue during shock. The viscera and the serous fluids became distinctly blue.

In shock loss of fluid occurs by vomiting and diarrhea, by edema and serous effusions, and by local loss of blood and fluid at the site of the injury when such injury has occurred. But no local loss of blood or fluid occurs when shock is produced by intravenous injections of bile or its salts, various drugs and poisons, histamine, peptone, bacterial products or by anaphylaxis. Any local loss of blood and/or fluid as in extensive trauma or burns, is a factor, but it is not the only factor. Its importance is proportional to the amount of blood and fluid lost.

Conclusions. Intraperitoneal or intravenous injections of bile or of sodium glycocholate produce the shock syndrome characteristically in dogs. This is accompanied by hemo-concentration as in shock otherwise produced. Sublethal degrees of shock follow sublethal doses of the agents mentioned. These may recover or may result in pulmonary edema, pneumonia and subsequent death. The postmortem findings are the same as those following shock otherwise produced. These are: marked capillary and venous engorgement of the lungs, serosa, gastro-intestinal mucosa, liver and kidneys; edema of lungs, gastro-intestinal mucosa, and serous effusions; anemia of the splenic substance. The evidence indicates that bile or its salts cause acute injury to the walls of capillaries and venules. This results in atony and increased permeability, whereby a disparity develops between blood volume and volume-capacity of the vascular system. Such a disparity manifests itself in the shock syndrome.

8779 C

Fermentation of Pyruvic Acid by Bacteria of the Colon-Aerogenes Group.*

MILO MICKELSON, HOWARD REYNOLDS AND C. H. WERKMAN.

From the Department of Bacteriology, Iowa State College, Ames.

In view of the intermediary rôle assigned to pyruvic acid, an understanding of its behavior is of importance in formulating schemes of dissimilation for bacteria of the colon-aerogenes group. The anaerobic dissimilation of pyruvic acid by organisms of this group is discussed in the present work.

Aubel[1] and de Graff and Le Fevre[2] report the products of the fermentation of pyruvic acid by *Escherichia coli* to be acetic and formic acids, carbon dioxide and hydrogen. Acetaldehyde is reported to be an intermediate.

Reynolds,[3] fermenting glucose with *Esch. coli* under conditions of forced aeration, recovered much of the carbon as pyruvic acid with corresponding decreases in lactic acid. The rise and fall of pyruvic acid during the fermentation of glucose by *Citrobacter freundii* further substantiates its position as an intermediate.

Cells were prepared by centrifuging 24-hour cultures, washing once with physiological salt solution, and resuspending in enough solution to make a paste. A medium consisting of 10 gm. glucose, 8 gm. peptone, 5 gm. disodium phosphate per liter gave good growth. The medium was sterilized at 20 pounds for 30 minutes. Phosphate buffer was sterilized separately and added to the glucose and peptone after autoclaving. Incubation took place at 30°C. The cultures were identified strains used for several years in this laboratory: *Aerobacter indologenes,* strain 23B, isolated and described by Burkey[4]; *Esch. coli,* American Type Culture Collection No. 26; and *C. freundii,* strain described by Werkman and Gillen.[5]

The dissimilations were conducted anaerobically in 300 ml. of 0.1M phosphate buffer at pH 6.2. The flask and absorption-train were flushed with oxygen-free nitrogen before inoculation.

Carbon dioxide was absorbed in Bowen potash bulbs and weighed.

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* Supported in part by Industrial Science Research Funds of Iowa State College.

[1] Aubel, E., *Bull. de la Soc. Chim. Biol.*, 1924, **6**, 288.

[2] de Graff, W. C., and Le Fevre, A. J., *Biochem. Z.*, 1925, **155**, 313.

[3] Reynolds, H., unpublished thesis, Iowa State College, 1935.

[4] Burkey, L. A., *Iowa State Coll. J. Sci.*, 1928, **3**, 57.

[5] Werkman, C. H., and Gillen, F. G., *J. Bact.*, 1932, **23**, 167.

An aliquot of the fermentation-liquor was acidified to congo red with 25% H_2SO_4, and residual carbon dioxide was determined by heating and aerating under a reflux condenser for an hour. Hydrogen was determined with the Hempel buret.

Before analysis of the liquor, the cells were removed by adding 1 ml. of phosphotungstic acid (20% in 5% H_2SO_4) per 20 ml. of medium and centrifuging. Acetic and formic acids were determined according to Osburn, Wood, and Werkman[6] on the steam distillate of an aliquot. Lactic acid was determined by the method of Friedemann and Kendall[7] and 2,3-butylene glycol according to Brockmann and Werkman.[8]

Purity of the cultures was established by examination of gram-stained smears before and after fermentation.

Controls of cells and buffer only were run and the small yields of products from cells were subtracted.

TABLE I.
Dissimilation of Pyruvic Acid by *Esch. coli.**

	Pyruvic	Acetic	Lactic	CO_2	H_2
Initial	65.46				
Final	0	51.26	15.60	47.46	34.41

*Products expressed as millimoles per liter.

The results indicate (Table I) pyruvic acid to be a precursor of lactic acid. The difference in yield of carbon dioxide and hydrogen is equivalent to that used in reducing the pyruvic acid to lactic acid. The results suggest the following breakdown:

(1) $CH_3COCOOH + HOH \rightarrow CH_3 . C . (OH)_2 . COOH \rightarrow CH_3COOH + HCOOH$
(2) $HCOOH \rightarrow H_2 + CO_2$

Although de Graff and Le Fevre[2] accept acetaldehyde as an intermediate in the decomposition of pyruvic acid by organisms of the colon-typhoid group, subsequently they report the products as acetic and formic acids, carbon dioxide and hydrogen. Whetham[9] presents evidence for the same products. *Esch. coli* possesses an active formic acid-splitting enzyme. Hydrogen thus liberated serves to reduce suitable acceptors such as pyruvic acid. *Esch. coli* lacks the enzymic equipment necessary to use the hydrogen to reduce 2 molecules of acetic acid to 2,3-butylene glycol through acetylmethylcarbinol. The case is different with *Aerobacter indologenes.*

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[6] Osburn, O. L., Wood, H. G., and Werkman, C. H., *Ind. and Eng. Chem.* (Anal. Ed.), 1933, **5**, 247

[7] Friedemann, T. E., and Kendall, A. I., *J. Biol. Chem.*, 1929, **82**, 23.

[8] Brockmann, M. C., and Werkman, C. H., *Ind. and Eng.. Chem.* (Anal. Ed.), 1933, **5**, 206.

[9] Whetham, M. D., *Aust. J. Exp. Biol. and Med. Sc.*, 1927, **4**, 35.

TABLE II.
Dissimilation of Pyruvic Acid by *Citrobacter freundii.**

	Pyruvic	Acetic	Lactic	Formic	CO_2	H_2
Initial	48.16					
Final	0	39.89	6.00	14.51	32.09	28.71

*Millimoles per liter.

The results (Table II) with *C. freundii* suggest the same mechanism of breakdown as for *Esch. coli.* In this fermentation, however, not all of the formic acid was decomposed. Six millimoles of pyruvic acid have been reduced to lactic acid; the remainder oxidized to acetic acid, and formic acid which is split into CO_2 and H_2. *C. freundii* is unable to bring about the reduction of acetic acid to 2,3-butylene glycol.

TABLE III.
Dissimilation of Pyruvic Acid by *Aerobacter indologenes.**

	Pyruvic	Acetic	2,3-B.G.	AMC	CO_2	H_2
Initial	50.60					
Final	0	28.8	9.4	—	48.54	24.85

*Millimoles per liter.

The differentiating characteristic of *Aerobacter indologenes* is its property of condensing and reducing acetic acid to 2,3-butylene-glycol through acetylmethylcarbinol. This utilization of hydrogen results in the ratio of $CO_2:H_2 = 2$ instead of 1 which is characteristic of *Esch. coli.* Thus it is apparent that the gas-ratio and the Voges-Proskauer test (for amc.) so widely used in differentiating the genera, are expressions of the same basic mechanism of dissimilation.

In Table III, 18.8 mM of acetic acid (or a precursor) have been reduced to 2,3-butylene-glycol requiring 28.2 mM hydrogen.

8780 C

Effect of Adrenotropic Hormone on the Natural Resistance of Hypophysectomized Rats.

DAVID PERLA.

From the Laboratory Division, Montefiore Hospital, New York.

In the present communication the protective effect of the adrenotropic fraction of anterior hypophysis on the natural resistance of hypophysectomized rats to histamine poisoning is reported.

We were unable to obtain the adrenotropic fraction prepared according to Collip's method[1] and as a source of this material we used an acid extract of the anterior hypophysis boiled for 20 minutes. Such a heated acid extract still contains adrenotropic activity, but the thyrotropic hormone and gonadotropic and growth factors are destroyed (Spence[2]). Preliminary experiments showed that such heated extracts still possess varying degrees of adrenotropic effect in hypophysectomized rats.

The acid extract of dried anterior hypophysis was prepared by the method of Loeb.[3] Ten grams of the powder was extracted with 100 cc. of ½% acetic acid overnight. The fluid was brought to pH 6.6, centrifuged and then brought to pH 7.8 and filtered through a Seitz filter. The heated fraction was prepared by suspending the extract in boiling water for 20 minutes.

Seventy-seven rats of Wistar stock, free from *Bartonella muris*, were used in these experiments. Of these, 14 were normal unoperated controls; 19 were completely hypophysectomized but untreated; 23 were hypophysectomized and injected with 1 cc. of acid extract of anterior hypophysis daily from the day of operation to the day of the test; 21 were hypophysectomized and injected daily with 1 cc. of the heated acid extract of anterior hypophysis.

Ten days after the operation the rats were injected intraperitoneally with amounts of histamine varying from 300 to 1600 mg. per kg. of body weight as indicated in Table I.

The minimal lethal dose of histamine in the control rats of Wistar stock* was about 1400 mg. per kg. The hypophysectomized rats untreated were killed in all instances by amounts of histamine greater than 400 mg. per kg. and only half the number of rats survived this dose. Hypophysectomized rats repeatedly injected

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[1] Anderson, E. M., Thomson, D. L., and Collip, J. B., *Lancet*, 1933, **2**, 347.

[2] Spence, A. W., *Saint Bartholomew's Hospital Reports*, 1935, **67**, pt. 6.

[3] Loeb, L., and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 490.

*This was somewhat higher than in the rats of our stock previously used.

TABLE I.

The Effect of Repeated Injections of Unheated and Heated Acid Extracts* of Anterior Hypophysis on the Natural Resistance of Hypophysectomized Rats to Histamine Poisoning.†

No. of rats	Amt. histamine per kg. body weight, mg.	No. of rats survived	No. of rats died
Hypophysectomized rats treated with acid extract of anterior hypophysis.			
3	300	3	0
3	400	3	0
2	500	2	0
2	600	1	1
4	700	2	2
4	800	3	1
4	900	2	2
1	1000	0	1
Hypophysectomized rats treated with a heated acid extract of anterior hypophysis (adrenotropic fraction).			
2	300	2	0
3	500	2	1
4	600	4	0
5	700	3	2
5	800	0	5
2	900	0	2
Hypophysectomized rats untreated.			
2	200	1	1
3	300	1	2
7	400	2	5
3	500	0	3
2	600	0	2
2	700	0	2
Normal control rats.			
2	1000	2	0
3	1100	2	1
4	1200	4	0
2	1400	1	1
2	1600	0	2
1	1800	0	1

*The extract was injected during a period of 10 days after operation in amounts of 1 cc. per rat per day.

†All the rats were 3 to 4 months of age at time of hypophysectomy.

with an acid extract of desiccated anterior hypophysis from the day of operation survived in some instances 900 mg. of histamine per kg. When treated with the adrenotropic fraction (the heated acid extract) hypophysectomized rats survived as great an amount as 700 mg. per kg. All succumbed to amounts in excess of 700 mg. per kg. of body weight.

The heated acid extract was not as effective as the unheated extract in raising resistance. This may be due to the fact that boiling partly destroys the adrenotropic fraction or that other hormonal factors present in the acid extract may influence the resistance of the hypophysectomized rat.

Since the heated acid extract is apparently free from all factors except the adrenotropic hormone it is probable that the protective effect of the acid extract is due to the adrenotropic fraction.

The evidence reported in this paper further supports the hypothesis that the decrease in natural resistance in the rat that follows hypophysectomy is in part an expression of insufficiency of the suprarenal cortex, due to withdrawal of the adrenotropic hormone.

The experimental evidence for this hypothesis may be summarized as follows:

(1) The removal of the suprarenal glands of rats is followed by a marked depression in the natural resistance to various toxins, poisons and bacterial and protozoan infections.† The depression is associated to a greater degree with the loss of cortical than with that of medullary substance. This conclusion is based on the fact that the administration of epinephrine to suprarenalectomized rats influences the resistance only to a slight degree,[5] whereas the administration of the suprarenal cortical hormone may raise the resistance almost to the normal level.[6]

(2) The natural resistance of completely hypophysectomized rats to histamine poisoning is depressed during a period of one to ten weeks after operation and this decrease in resistance is associated with involutional changes of the suprarenal gland, such as hemorrhage into or atrophy of the inner zones of the cortex. Rats in which the posterior lobe and a large portion of the anterior lobe are removed show a similar drop in resistance and atrophic changes in the suprarenal cortex occur, but when a large fragment of anterior lobe remains no depression in resistance to histamine occurs and the suprarenal glands are normal.[7, 8]

(3) Repeated injection of large amounts of suprarenal cortical hormone raises the natural resistance of totally hypophysectomized adult rats to histamine poisoning.[9] This is particularly marked during the first 2 weeks after operation, the period during which the most severe depression in natural resistance occurs.

(4) The natural resistance of hypophysectomized rats can be raised by the administration of (a) emulsions of fresh anterior hy-

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† For a review of this subject, see Perla and Marmorston.[4]

[4] Perla, David, and Marmorston, J., *Arch. Path.*, 1933, **16**, 379.

[5] Perla, D., and Marmorston-Gottesman, J., *Am. J. Physiol.*, 1929, **89**, 152.

[6] Perla, D., and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1022.

[7] Perla, D., and Rosen, S. H., *Arch. Path.*, 1935, **20**, 222.

[8] Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 655.

[9] Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 797.

pophysis of cattle,[10] (b) acid extracts of dried anterior hypophysis rich in adrenotropic factor, and (c) heated acid extracts of anterior hypophysis containing only active adrenotropic hormone (present communication).

The decrease in natural resistance that follows hypophysectomy in the rat to a poison such as histamine may be due to withdrawal of the adrenotropic hormone and the consequent insufficiency of the suprarenal cortex.

8781 P

Functional Pituitary Grafts in Rats.

R. O. GREEP. (Introduced by Frederick L. Hisaw.)*

From the Biological Laboratories, Harvard University.

Recent experiments by Hohlweg and Junkmann,[1] Haterius, Schweizer and Charipper,[2] and Hill and Gardner[3] have indicated quite conclusively that pituitary tissue will function as a graft.

It is the purpose of this report to present summarily a series of experiments in which the empty sellae of hypophysectomized 28-day-old male and female rats were utilized immediately after the operation as sites for grafting pituitary tissue. The types of grafts included: 1. male hosts bearing (a) their own pituitaries as grafts and (b) the pituitaries of 28-day-old female rats; 2. female hosts bearing (a) their own pituitaries as grafts (b) the pituitaries of other 28-day-old female rats (c) the pituitaries of 1 to 5 newborn male rats; (d) the pituitaries of male rats 20 to 40 days old. Hosts and donors were taken from an intensively inbred strain of rats. In a group of 37 such hosts (5 males and 32 females) which were observed for 14 weeks, 73% takes occurred as judged by marked body growth and attainment of a functional sexual capacity.† The growth curves of these animals ascended much more gently and flattened out at one-half to two-thirds the heights of similar curves for normal non-hypophysectomized controls.

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[10] Perla, David, PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 121.

* Aided in part by a grant from the National Research Council, Committee on Problems of Sex, administered by Frederick L. Hisaw.

[1] Hohlweg, W., and Junkmann, *Klin. Wchnschr.*, 1932, **11**, 321.

[2] Haterius, H. O., Schweizer, M., and Charipper, H. A., *Endocrinology*, 1935, **19**, 673.

[3] Hill, R. T., and Gardner, W. U., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 78.

† In a group of 70 operative controls 69 proved to be completely hypophysectomized as determined by growth stasis, severe cachexia and macroscopic examination of the sella.

The sexual development and history of the grafted females were similar in all groups regardless of the source of the transplanted tissue. Vaginal smears and exploratory examinations of the ovaries established the fact that typical cyclic oestrous phenomena occurred. The cycles in different animals varied in length from 4 to 14 days, the majority having a 5- to 7-day rhythm. The elongated cycles were due in every instance to a prolonged dioestrous interval. Ovulation was spontaneous and the resultant corpora lutea appeared normal. Thirteen pregnancies were recorded, 8 occurring among the females bearing male pituitaries. Living young were delivered at term and lactation was normal.

Females bearing a single newborn male rat pituitary grew very slowly and never became sexually mature while 5 such pituitaries per host proved very effective.

Reproductive function was sustained in males by grafts of either male or female anterior lobe tissue in the sellae, thus confirming the observation of Hill and Gardner,[3] who found that pituitary tissue of the female mouse grafted into the testis of a male would maintain the male system.

8782 C

Influence of Potassium and Calcium on Motor Discharges.

R. W. GERARD AND H. W. MAGOUN.

From the Department of Physiology, University of Chicago, and the Institute of Neurology, Northwestern Medical School.

Physico-chemical factors within neurones must be important in controlling their activity, both spontaneous and evoked. Impinging nerve impulses are the main secondary control in the latter case; the condition of the fluid milieu of the cells in the former. The prolonged motor discharges which give maintained rebound postures following stimulation of the deep cerebellar nuclei[1] are a favorable case of evoked activity grading slowly into spontaneous activity. Since K+ and Ca++ are able respectively to increase and decrease potentials in the brain,[2] these ions were tested for effects on intensity and duration of the motor sequelae of cerebellar stimulation.

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[1] Magoun and Hare, *Am. J. Physiol.*, 1936, **116** (in press).

[2] Gerard and Dubner, *Arch. of Neurol.*, 1936, **36** (in press).

A stimulating electrode was placed by the Horsley-Clark instrument in the nucleus fastigius of cats under light nembutal anesthesia. A stimulus was chosen to give a good, but not maximal response. During stimulation (up to 3 sec.) the crossed foreleg is flexed at the elbow; to pass into strong extension on cessation of the stimulus. This after-extension was timed and its intensity judged semiquantitatively by feel. After several repetitions had yielded constant times, 1 or 2 cc. of isotonic KCl, $CaCl_2$ or Na citrate were injected into the carotid artery and observations continued within 30 seconds. In each of 3 tests with K, responses were prolonged beyond those earlier or later by an average of 230%. (The first 3 responses in each series are averaged.) Likewise, Ca++ consistently decreased response duration on the average by 51%. Sodium citrate increased durations 10 to 20%. Intensity fairly paralleled duration, being increased with K+ and decreased with Ca++.

TABLE I.

Condition	Duration of Extension (sec.)	
Before injection	5.5, 4.5, 4.5, 4.2	
KCl—2 cc.	10.5, 11.5, 16.5, 8.0, 5.5, 3.5, 3.5	
15 min. later	6.8, 5.0, 4.4, 4.2, 4.2	
KCl—2 cc.	13.7, 13.3, 13.0, 6.0, 6.5, 6.0, 6.0	
5 min. later	4.0, 5.0, 3.8	
15 ’’ ’’	5.0, 3.5, 3.8	
Before Ca	23.5, 15.0, 20.0 — — —	
$CaCl_2$—2 cc.	10.0, 10.8, 8.5 — — —	
10 min. later	16.0, 18.5, 18.0 — — —	
	Aver. responses	
	Exp. I	Exp. II
Control (before and after)	5.2	24.5
Calcium	2.9	13.0
Control	4.9	17.4
Potassium	13.1	25.2
Control	6.3	16.6
Citrate	8.3	17.9

In any series of tests under "control" conditions the first 1 or 2 responses, after a rest, may be shorter than later ones; and still later responses are again shorter. After salt injections, in each case the third test, about 1 minute after the injection, gave the extreme value—longest after K or citrate, shortest after Ca. Table I gives 2 single series and averages.

8783 C

Influence of Potassium Concentration on Temperature of Cold Standstill in the Turtle Heart.

George H. Zwikster and T. E. Boyd.

From the Department of Physiology and Pharmacology, Loyola University School of Medicine.

In 1934 Ets and Boyd[1] reported that frog nerves, soaked in modified Ringer solutions containing an excess of potassium, are thereby rendered abnormally susceptible to block by local cooling. Nerves kept in ordinary Ringer solution invariably continued at low temperatures to conduct until they were frozen.

We have investigated the effects of varied concentrations of potassium on the behavior of the turtle heart at low temperatures. The excised atria of *Pseudemys elegans,* with sinus attached, were suspended in an aerated bath containing the solution to be tested. The preparations were left in the solution for 30 minutes at constant temperature—in winter at 20°C., in summer at 28°—and then gradually cooled. The temperature of stoppage was determined from the graphic record made with a light isotonic lever. Both sinus and atrial beats ceased abruptly, and not by gradual fading. Sino-atrial block appeared first, but as a rule only a few sinus beats occurred thereafter.

Unbuffered Ringer solutions were used. They were modified

TABLE I.

Solution, % KCl	No. and type of preparations	Stoppage temp. (sinus) ° C.
Frog Ringer NaCl .650 KCl .014 $CaCl_2$ .012	12, summer	Below 1
Same, KCl .056	20, ”	5 to 8
Turtle Ringer NaCl .700 KCl .040 $CaCl_2$ .025	8, ” 20, winter	4 to 6 0 or lower
Same, KCl .02	5, summer	Below 1
Same, KCl .12	3, ” 9, winter	14, 14, 6 3 to —1
Same, KCl .16	2, summer 4, winter	28, 23 10, 10, 10, 11

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[1] Ets, Harold N., and Boyd, T. E., *Am. J. Physiol.*, 1934, **108**, 129.

by changing the concentrations of KCl and NaCl in opposite directions, keeping the osmotic pressure constant.

It is evident that the low limit of temperature for spontaneous beats varies according to the potassium content of the solution in which the heart has been kept. Martin[2] found this to be true of the beats of the turtle ventricle, but since he stated that the auricles were relatively resistant to cooling, he was presumably dealing with an atrio-ventricular block rather than a complete stoppage.

The relatively high susceptibility of summer preparations, indicated in the table, is apparently due to an actual seasonal variation in the condition of the tissue, and not to the higher initial temperature from which the cooling was started. Preliminary immersion at 20° instead of 28° does not render such a preparation appreciably more resistant to cooling. Regardless of seasonal variations and initial temperature, however, any preparation can be cooled to 0° without stopping, if it has been kept for a time in a sufficiently low concentration of potassium.

The effect of the higher concentrations of KCl is reversible. A preparation kept in Ringer solution containing 0.160% KCl shows, on repeated cooling, a progressively higher temperature of stoppage until beats finally cease at room temperature. On transferring the preparation to normal frog or turtle Ringer the process is reversed, beats eventually continuing at 0°. Balancing the original solution, by adding a corresponding excess of calcium, is not effective in causing such recovery.

8784 P

Cytological Studies on Experimental Rat Tumors.

E. von Haam, H. Alexander and H. Beard.

From the Departments of Pathology and Bacteriology and Biochemistry, Louisiana State University Medical Center, New Orleans, Louisiana.

The present investigation is the first attempt at an experimental approach to the problem of nucleolus size in cancer cells. In a previous publication, von Haam and Alexander[1] reported studies on nuclear and nucleolar measurements in fixed and unfixed cells of malignant tumors in man. After examining 10,000 cells, 5,000 of which were taken from non-malignant tissues and served as controls, the authors came to the conclusion that the number of excessively large nucleoli encountered in malignant tissues was sufficient to produce a distinct difference in the nuclear-nucleolar ratio between benign and malignant lesions based on average values of at least 100 cells to a specimen.

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[2] Martin, E. G., *Am. J. Physiol.*, 1904, **11**, 370.

[1] von Haam, E., and Alexander, H. G., *Am. J. Clin. Path.*, 1936, (in press).

The material for the present investigation was experimental tumors produced in the albino rat by means of radiation of the animals with ultraviolet rays over a long period. With this method, which has been described in detail by Roffo; Beard, Boggess and von Haam[2] obtained in 40% of their animals malignant tumors of various histological types which proved fatal to the animals. The technic of measurements of the nuclei and the nucleoli in the experimental tumors was identical with that described by von Haam and Alexander with the exception that only fixed tissue cells were used for the determinations, since it had been shown that proper fixation has no appreciable influence on the nuclear-nucleolar ratio.

Satisfactory selective staining of the nucleoli was obtained with Victoria blue or with Regaud's iron hematoxylin. Toluidine blue which had been used extensively in measurements on unfixed cells, did not give satisfactory results with fixed tissues. Twelve tumors in albino rats were examined—there were eight carcinomas of the ear, one carcinoma of the nose and three sarcomas of the eye. Skin from healthy animals, taken from regions corresponding to the location of the malignant tumors, and liver and kidney epithelium of some of the tumor animals served as controls. The results obtained are summarized in Table I.

The nuclear-nucleolar ratio of the cells examined in the malignant

TABLE I.

Prot. No.	Type of tissue examined	Nuclear Nucleolar Ratio (100 cells)	Prot. No.	Type of tissue examined	Nuclear Nucleolar Ratio (100 cells)
1T	Carcinoma of ear	12.4:1	6T	Epithelium, kidney	16.5:1
2T	" " "	11.7:1	5C	" skin	23.3:1
3T	" " "	13.5:1	4T	" liver	18.6:1
4T	" " "	13.4:1	15C	" skin	19.0:1
5T	" " "	8.85:1	1T	" kidney	16.5:1
6T	" " "	10.8:1	6T	" liver	20.8:1
7T	Sarcoma " eye	10.3:1	12C	" skin	27.4:1
8T	Carcinoma " ear	9.1:1	19C	" "	25.3:1
9T	" " nose	10.1:1	6T	" "	15.3:1
10T	" " ear	10.9:1	17C	" "	29.7:1
11T	Sarcoma " eye	11.3:1	21C	" "	27.5:1
12T	" " "	12.9:1	6C	" "	25.5:1

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[2] Beard, H., Boggess, T. S., and von Haam, E., *Am. J. Cancer*, 1936 (in press).

lesions ranged between 8.85:1 and 13.5:1, while the cells taken from normal tissues of the control animals and the tumor animals ranged between 15.3:–1 and 29.7:1. Cytological study of the malignant cells showed not only large nucleoli but also great irregularity in the shape, staining properties and number of nucleoli present in one nucleus—factors which we previously stressed as characteristic of the malignant cells from human tumors. The difference between malignant and non-malignant cells as regards nuclear-nucleolar ratio is more marked in our series of experimental tumors than it was in our series of human lesions which may partly be explained by the fact that the controls in this study were healthy tissues not the seat of inflammatory changes.

Conclusions. Study of experimental tumors produced by ultraviolet rays in the albino rat revealed low average values for the nuclear-nucleolar ratio of groups of 100 cells. The nucleolus from experimentally produced malignant tumor cells was found to vary greatly in size and number as it does in malignant cells from human lesions.

8785 C

Copper Hematoxylin, a Stain for the Acidophils of the Human Hypophysis.

A. T. Rasmussen.

*From the Department of Anatomy, Medical School, University of Minnesota, Minneapolis.**

Cushing's[1] announcement that a pleuriglandular syndrome, designated by him as pituitary basophilism, was associated with basophilic tumors of the anterior lobe, attracted special attention to the basophils of the human hypophysis, including those that invade the posterior lobe. The latter variety, arising from the indifferent-staining cells of pars intermedia,[2] have been brought into prominence in connection with hypertension.[3]

Similarity in morphology and staining properties of the basophilic cells of the anterior and those in the posterior lobe has been been

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* Aided by a grant from the research fund of the Graduate School, University of Minnesota.

[1] Cushing, H., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 137.

[2] Rasmussen, A. T., *Am. J. Anat.*, 1930, **46**, 461.

[3] Cushing, H., *Am. J. Path.*, 1934, **10**, 145.

advanced as evidence that the latter have migrated from the anterior lobe. Dayton[4] calls attention to the similarity in their behavior in various physiological and pathological conditions. Of those who have noted the great similarity in staining reaction, Kasche,[5] Biedermann[6] and Burgdorf[7] may be mentioned.

MacCallum, Futcher, Duff and Ellsworth,[8] in connection with a typical case of Cushing's syndrome with a tumor in the anterior lobe, decided that copper hematoxylin stained the basophils of the anterior lobe but not those in the posterior lobe. Since the tumor cells did not stain with this technique and had morphological features somewhat similar to the cells of pars intermedia, they concluded that the tumor probably arose from pars intermedia, which, of course, arouses new interest in this part of the hypophysis. At the recent meeting of the Association for the Study of Internal Secretion at Kansas City, Lisser reported a certain amount of recovery in a patient with Cushing's disease after partial removal of a hypophyseal adenoma. That the tissue removed at operation was from a pars intermedia neoplasm, was at least partly inferred from the staining reaction with copper hematoxylin and the assumption that this technique stained the basophils of the anterior lobe, but not those that arise from pars intermedia, as announced by MacCallum, *et al.*[8]

The facts are that copper hematoxylin, as given in McClung's Microscopical Technique[9] or by substituting potassium dichromate for neutral potassium chromate, stains the acidophils rather than the basophils of either lobe. Bailey,[10] years ago, grouped Weigert's myelin sheath stain (a copper hematoxylin method) with the more or less specific stain for the acidophilic granules. Copper hematoxylin is also a mitochondrial stain and the difficulty in separating mitochondria from the acidophilic granules in the hypophysis is proverbial.

Sections from human hypophyses fixed in formalin, Bouin's, Helly's, Regaud's, Zenker's and Zenker-formol (half the acetic acid replaced by formalin) fluid, all give essentially the same results. We have selected specimens that happened to have groups of cells that could be identified without question. One section of a pair of adjacent 5μ sections was stained by the well-known Mallory's acid fuchsin-aniline blue-orange G method, which has been used extensively on the hypophysis, and the other one by the copper hematoxylin method. Sections have been stained in copper hematoxylin and then counter-stained with other acidophilic stains and no acidophils were seen that were not already stained by copper hematoxylin. We have drawn particular cells in colors under high power and decolorized and then stained with copper hematoxylin. In all instances it is perfectly clear that the basophils of the anterior lobe, like those in the posterior lobe, are not stained in fully differentiated copper hematoxylin slides, while the acidophils are. The basophils may have a darker grayish-brown tone than the chromophobes, but this is not sufficiently marked to serve as a method of separating basophils from the chromophobes. All acidophils do not hold the stain with equal tenacity. This is more or less true of all methods and applies to positive staining (by other methods) of basophils as well.

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[4] Dayton, T. R., *Ztschr. f. Anat. u. Entwcklngsgesch.*, 1926, **81**, 359.

[5] Kasche, F., *Ztschr. f. mikr-anat. Forschung*, 1926, **6**, 191.

[6] Biedermann, H., *Virchow's Arch. f. path. Anat. u. Physiol.*, 1927, **264**, 217.

[7] Burgdorf, A. L., *Endokrinologie*, 1935, **16**, 148.

[8] MacCallum, W. G., Futcher, T. B., Duff, G. L., and Ellsworth, R., *Bull. Johns Hopkins Hosp.*, 1935, **56**, 350.

[9] McClung, C. E., *et al.*, 1929, p. 203.

[10] Bailey, P., *J. Med. Research*, 1921, **42**, 349.

The basophils can usually be identified among the unstained cells in the copper hematoxylin method because of their greater vacuolation. Having sectioned serially the hypophysis of three cases of pituitary basophilism, we can corroborate the findings of Crooke,[11] who described hyaline areas of various sizes in the cytoplasm of the basophils of the anterior lobe and to some extent also of the posterior lobe. This modified cytoplasm also serves to identify the basophilic cell among those not stained with copper hematoxylin. While the basophils of the posterior lobe are more resistant to these changes than are those of the anterior lobe, some of them show exactly the same change, giving further proof of the great similarity between the basophils in the two locations.

It is, therefore, concluded that copper hematoxylin is not a stain that will differentiate between the basophils of the anterior lobe and those of the posterior lobe, by staining the former and not the latter, because neither retains the stain. On the contrary, as has been recognized by others, it is a good positive stain for the acidophils of the human hypophysis.

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[11] Crooke, A. C., *J. Path. Bac.*, 1935, 339.

8786 P

Effect of Varying pH on Toxic Effect of Bile Salts on the Normal Gall Bladder.*

Hans G. Aronsohn and Edmund Andrews.

From the Department of Surgery, The University of Chicago.

On injection of bile salts into the dog's gall bladder we obtained, as reported in previous papers,[1, 2] marked changes of the gall bladder wall, which on gross and microscopic examination, closely resemble those found in human cholecystitis. In the following study, these findings are subjected to thorough criticism because of their importance in explaining the origin of human cholecystitis.

First, a sufficient number of experiments, more than 70, have been performed to allow us to draw conclusions. Secondly, the technique used (retrograde catheter-cannula) has been described in a previous paper[3] and an ample series of controls has been run. Furthermore, the unimportant rôle played by bacterial infection is seen from previous work, as injection of overwhelming numbers of bacteria into the dog's gall-bladder causes little or no reaction on the gall-bladder wall.[4]

A possible objection against our contention that the cholecystitis obtained had been caused by the toxicity of the bile salts, is that these bile salts might bring about their effect by changing the pH of the gall bladder bile, thus converting the bile itself to a toxic substance. There are two ways of controlling this. One would be to change experimentally the pH of the gall bladder bile by adding acids or alkali and to observe the effect this change might have in the gall bladder wall. The other consists of injecting bile salt solution after adjusting its pH to that of gall bladder bile. Both methods have been applied. Large amounts of strongly acid or alkaline solutions are necessary to overcome the buffering action of bile. Death of the animal and total gangrene of the gall bladder follows the injection of 5 cc. of glacial acetic acid into the dog's gall bladder. The pH after adding this acid to the gall bladder bile and thoroughly

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* Work done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Aronsohn, H. G., and Andrews, E., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 87.

[2] Andrews, E., and Aronsohn, H. G., *loc. cit.*

[3] Aronsohn, H. G., and Andrews, E., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1629.

[4] Aronsohn, Hans G., *Am. J. Surg.*, 1936, New Series **32**, 18.

mixing was about 2. Similarly, total gangrene of the gall bladder wall was obtained by adding 10 cc. of Na_2CO_3 20% to the gall bladder bile with a final pH of 10. However, here death of the animal did not occur, and the effects were wholly local. The following detailed study shows the extent of the buffering potentiality of bile.

On aspirating the total gall bladder bile, washing the gall bladder with a citric phosphate buffer solution of pH 4 and leaving the gall bladder filled with this solution, in 2 experiments practically no change at all occurs in the gall bladder wall. In 2 other experiments, again the total gall bladder bile was aspirated and a citric phosphate solution of pH 3 left in the gall bladder. In one of these, the gall bladder, containing a colorless thin fluid, showed a moderate degree of acute cholecystitis. The other, however, was refilled with dark bile and revealed a normal gall bladder wall. It is quite obvious that the bile which had reentered the gall bladder through the hepatic duct by its buffering action had kept the acid mixture from developing an irritating action on the wall of the gall bladder.

The second series of experiments consists of the introduction of bile salts previously adjusted to the pH of the gall bladder bile into the dog's gall bladder and reveals similar results.

Various difficulties were encountered in performing these experiments. The first and chief difficulty was that adding of acid to a bile salt solution very easily causes precipitation of the bile acids. Special precautions are necessary to prevent this precipitation and keep the bile salts in solution. Furthermore, we have to consider that many acids, especially inorganic ones, if brought in contact with the gall bladder wall, themselves set up a reaction. We could overcome these difficulties by using for the adjustment a weak citric acid solution, which, as seen from controls, would not cause any change on the gall bladder wall even in higher concentration than that used. As an example the adjusting procedure for sodium desoxycholic is given as follows: 12 gm. sodium desoxycholic are dissolved in 100 cc. aq. dest. This solution which has a pH of 8.4, is heated to body temperature and 0.1 M citric acid added one drop at a time. The precipitate which occurs is dissolved by shaking. After the desired pH is reached (determined first roughly by a set of indicators) exact pH determination is done with Hastings colorimeter.

Table I shows the experiments with adjusted bile salts and the controls with citric acid.

Summary. 1. Strong acids or alkali of pH lower than 2 or higher

TABLE I.

Injected Solution	pH		Result
1. Sod. desoxychol. 10, 8%	pH after adjust. with 0,1 mol. citr. acid:	7,3	severe cholecystitis
2. ” ” 9%		7,2	” ”
3. Cholic acid 12, 6%		7,8	medium ”
4. ” ” ” 5%		7,4	” ”
5. Purif. B. S. 15%	pH without adjust.	5,7	moderate ”
6. ” ” 15%		5,7	” ”
7. Citric acid-NaOH	1 M. citric acid adjust. with NaOH 10%	7	normal gallbladder
8. ” ” ”		7,4	” ”

than 10, cause a gangrene of the gall bladder wall. 2. When an acid solution at pH 3 is injected into the empty gall bladder and bile through patent ducts is allowed to reenter the gall bladder, the buffering action of this bile is sufficient to convert the acid bile mixture into a harmless solution. 3. The toxicity of bile salts on the gall bladder wall is not changed by adjustment to the pH of gall bladder bile previous to injection. 4. Citric acid solution, in concentration even higher than that used for the adjustment, does not affect the gall bladder wall.

Conclusion. The toxic action of the bile salts on the normal gall bladder is not affected by alteration of the pH unless it exceeds the normal pH range of gall bladder bile.

8787 P

Relative Toxicity of Different Bile Salts on the Normal Gall Bladder.*

EDMUND ANDREWS AND HANS G. ARONSOHN.

From the Department of Surgery, The University of Chicago.

In a previous publication[1] we have shown that an experimental increase of the bile-salt concentration in the bile produces changes in the gall bladder wall similar to "human cholecystitis" Sixteen experiments had been performed, using "commercial bile-salts" (Armour and Company), and so-called "purified bile-salts", *i. e.*, the commercial variety freed from non-alcohol soluble impurities by alcohol precipitation. The latter proved the more effective preparation of the two. The technique employed consisted of the retrograde introduction of a fine ureter catheter into dog's gall bladders through an opening in the common duct and introduction of the bile-salt solution through this catheter.[2]

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* Work done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Andrews, E., and Aronsohn, H. G., *loc. cit.*

To elucidate this action further, we have made a more thorough investigation using different bile-salt fractions. The following results were obtained from 55 additional experiments:

The process of hydrolyzing increases rather than decreases the effectiveness of the bile salts. While a 20% solution causes a tremendous reaction on the gall bladder wall, a concentration as low as 8.7% still produces a marked effect. Two cases of bile peritonitis occurred in this group.

In a series of 13 experiments, a special study was made of Na desoxycholate. This proved to be the most powerful fraction, a concentration of 15% causing gangrene of the gall bladder wall, bile peritonitis, or even death, while even a concentration of 7.5% gave a marked reaction. Cholic and apocholic acid are slightly weaker in their actions than desoxycholic acid, apocholic being the stronger of the two. Positive reactions were obtained with 7% apocholic acid, while higher concentrations of cholic acid were necessary to produce similar changes. Glycocholic acid, on the other hand, has much less effect on the gall bladder wall than the fractions described above. An 8% solution yielded a normal gall bladder regularly, a reaction being obtained only with a 13% solution. Injection of taurocholic acid, obtained by evaporation of dog bile to about one-third of its volume, however, gave strongly positive results, *i. e.,* bile peritonitis and death accompanied by a marked change in the gall bladder wall. For the sake of completeness, we

TABLE I.

Fraction	No. of Exp.	Reaction
1. Desoxycholic acid	13	Severe reaction; gangrene of gallbl., bile peritonitis and death.
2. Apocholic and cholic acid	17	Same
3. Conc. dog bile (taurocholic acid)	4	Severe cholecystitis, bile peritonitis and death.
4. Hydrolyzed bile salts	6	Severe cholecystitis and death.
5. Purified " "	4	Same
6. Commercial " "	12	No death. Bile peritonitis and cholecystitis.
7. Glycocholic acid	9	Cholecystitis occasionally on high concentration, mostly normal gall bladder.
8. Decholin (dehydrocholic acid)	6	Normal gall bladder.

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[2] Aronsohn, H. G., and Andrews, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1629.

examined the action of Decholin (Dehydrocholic acid), which is used in the medical management of human cholecystitis. A 20% solution can be injected into the dog's gall bladder without producing a marked reaction in the gall bladder wall.

Table I shows the different fractions in decreasing order of their toxicity.

Grossly, the changes involve mainly the outer layers of the gall bladder wall. The usual findings were thickening of the wall, especially the serosa; thick fibrinous membranes, with fresh adhesions to the surrounding organs, duodenum and liver; and a serous or seropurulent exudate around the gall bladder. Bile peritonitis, either localized around the gall bladder, or a diffuse form without visible perforation of the gall bladder wall, was often seen. On microscopic examination, edema of the serosa was found with great regularity. In addition, the serosa, covered with a fresh fibrinous exudate, showed hyperemia, hemorrhages, dilatation of lymphatics, and round-cell infiltration. These changes were regularly found in those gall bladders which had been diagnosed as pathological on gross examination. There were no qualitative differences found in the appearance of the gall bladders after injection of the various fractions, the only differences being quantitative. There was, however, some difference in the character of the reaction produced by desoxycholic acid. In this case, apparently all of the layers were involved, there being leucocytic infiltration and hyperemia in the mucous membrane and muscular layer as well as in the serosa. It cannot be decided from the anatomical appearance whether this involvement of the inner layers was produced by secondary extension of the inflammatory process from the serosa to the muscular layer and mucous membrane, or by primary action on the latter.

We may conclude, in comparing the relative toxicity of different bile salts on the normal gall bladder wall, that desoxycholic acid has the strongest action.

8788 C

VI. Studies on Autodigestion. Digestion of Living Tissues by Trypsin.

H. Necheles, P. Levitsky and M. Maskin.

From the Gastro-Intestinal Laboratory, Department of Physiology, Michael Reese Hospital, and the Department of Physiology, University of Chicago.

Bernard demonstrated that the leg of a living frog can be digested by stomach juice, but not by trypsin solution.[1] Kirchheim's experiments in which he found digestion of frogs' legs with trypsin seem to be objectionable, on the grounds that in all probability his animals were moribund.[2] Subsequent authors have confirmed Bernard's original experiments (lately Dragstedt, Haymond and Ellis[3]*), and have further shown that erythrocytes,[4] the mucosa of the urinary bladder[5] and spermatozoa when exposed to trypsin,[2] and also that organs or tissues when implanted into the duodenum, were not digested (Necheles, Ling and Fernando,[6] and Dragstedt, Haymond and Ellis[3]). Boldyreff[7] was the first to suggest that trypsin may play a rôle in the genesis of peptic ulcer. This idea was received by later authors who attempted to produce gastric ulcer with trypsin,[8] but their evidence is not convincing.[9]

It is common knowledge that a number of proteins are digested only slightly, if at all, by trypsin, but that after a short exposure to pepsin-HCl, complete digestion with trypsin takes place. Based on this principle, it was determined to study the influence of pepsin on tryptic digestion. Living pithed frogs were exposed to pepsin-HCl first for a short period of time, which procedure of itself did not result in any visible digestion, and then immersed in a solution of trypsin, which as has been repeatedly demonstrated is not able to

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[1] Bernard, C., *Leçons de physiologie Expérimentale*, Paris, 1856, **2**, 408.

[2] Kirchheim, L., and Böttner, A., *Arch. exp. Path. u. Pharm.*, 1915, **78**, 99.

[3] Dragstedt, L. R., Haymond, H. E., and Ellis, J. C., *Arch. Surg.*, 1934, **28**, 232.

* The pertinent literature on peptic digestion has been reviewed in the previous paper of this series.[10]

[4] Matthes, M., *Münch. Med. Woch.*, 1902, **49**, 8.

[5] Fermi, C., *Centralblatt f. Bakter.*, 1910, **56**, 55.

[6] Necheles, H., Ling, T., and Fernando, F., *Am. J. Physiol.*, 1926, **79**, 1.

[7] Boldyreff, W., *Arch. f. d. ges. Physiol.*, 1907-8, **121**, 13.

[8] Stuber, B., *Z. f. Exp. Path. und Ther.*, 1914, **16**, 295.

[9] Stahnke, E., and Hsieh, T., *Z. f. d. ges. Exp. Med.*, 1927, **55**, 403.

[10] Maskin, M. H., Callahan, R., and Necheles, H., *Am. J. Dig. Dis. and Nutr.*, 1936, **3**, 174.

TABLE I.

No. of Series	No. of Frogs	Minutes of Alternating Exposure to:					
		HCl .36% Peps. 2%	Na_2CO_3 .25% Tryps. 2%	HCl .36% Peps. 2%	Na_2CO_3 .25% Tryps. 2%	HCl .36% Peps. 2%	Na_2CO_3 .25% Tryps. 2%
1	6	30	10 S	0	49 M		
2	9	20	18 S	0	120		
3	5	20	19 S	8	12 M		
4	5	8	14	7	15 S	9	10 M
		HCl .18% Peps. 2%					
5	5	25	11 S				
6	2	26 (22-30)	120 —				
		HCl .36% Peps. 2%					
7	5	23 (10-33)	120 —				
8	5	30 —	38 S	0 —	85 M		
9	5	20 —	43 S	0 —	120		
10	5	10 —	23	10 —	24 S	8 —	16 M
		HCl .18% Peps. 2%		HCl .18% Peps. 2%			
11	5	30 —	27 S	0	70 M (2)* 120 (3)*		
12	6	17 —	40 S				
13	5	18 —	35 S (4)*	13 —	24 M		
14	2	45 — (30-60)	60 —				
		HCl .36% Peps. 2%		HCl .36% Peps. 2%		HCl .36% Peps. 2%	
15	6	35 — (30-60)	65 (30-78) —				
16	1	30 —	30 —	5 —	30 —	5 —	25 —
17	3	—	114 (60-162)				

S = Skin Digestion. M = Muscle Digestion. Absence of both S and M denotes no digestion. Numbers without parenthesis denote averages; numbers in parenthesis denote range. * denotes number of frogs.

digest even the skin of a frog. The tests were conducted at a temperature of 38°C. by placing the containers in a constant temperature water bath. One hundred and two successful experiments were performed, but for lack of space only a number are reported below. *Ranae esculentae* with an average weight of 40 gm. were selected. Trypsin 1:300 and pepsin 1:10,000[†] were used in 2% solutions of HCl and Na_2CO_3 respectively. Of the former, 2 concentrations were used, 0.36% (N/10) and 0.18% (N/20), of the latter only 0.25%. The legs and one-third of the thighs of the frogs were immersed in this solution. The thorax was opened at the end of each experiment for observation of heart activity, and only such experiments in which the heart beat was normal at the termination of the test were accepted.

Our results can be grouped into 5 categories:

A. The influence of pepsin-HCl on tryptic digestion time,

B. The influence of HCl alone on tryptic digestion time,

C. A control series using pepsin-HCl followed by 0.25% Na_2CO_3.

D. A second control series using HCl alone followed by 0.25% carbonate, and

E. The negative effect of trypsin alone.

A. Exposure to pepsin-N/10 HCl for 30 minutes followed by trypsin resulted in skin digestion in 10 minutes, and muscle digestion in 49 minutes (Series 1). When the pepsin-HCl exposure was diminished to 20 minutes, tryptic digestion of the skin took place in 18 minutes, and no muscle digestion could be observed after 120 minutes (Series 2). But if an additional exposure of 8 minutes of pepsin-HCl was allowed once skin digestion had commenced, we obtained muscle digestion in the trypsin in 12 minutes, giving a total of 28 minutes in the pepsin and 31 in the trypsin (Series 3). In series 4, three repeated exposures of 8, 7 and 9 minutes in pepsin-HCl alternated with 14, 15 and 10 minutes in trypsin respectively. Skin digestion occurred during the second tryptic exposure, and muscle digestion during the third. Thus, if 24 minutes of pepsin-HCl were distributed over 3 periods, we were able to demonstrate muscle digestion in the trypsin in a total of 39 minutes, whereas it required 59 minutes to achieve the same end following a single initial exposure of 30 minutes to pepsin-HCl (Series 1). When the trypsin was preceded by pepsin-N/20 HCl for 25 minutes, the former digested the skin in 11 minutes (Series 5).

[†] We are obliged to Dr. D. Klein, Wilson Laboratories, for supply of part of the enzymes.

B. Comparing series 8-13, in which the trypsin was preceded by HCl alone with Series 1-5, it is evident that tryptic digestion of both skin and muscle is much slower than when pepsin is used in conjunction with the HCl. Here too we can demonstrate that repeated alternating exposures are more effectual than an equal single exposure (compare Series 10 with 8, and 13 with 11).

C. In series 6 and 7 frogs were first immersed in pepsin-HCl for 26 and 23 minutes respectively, followed by 0.25% Na_2CO_3 alone for as long as 120 minutes without any trace of digestion.

D. As might be expected, primary exposures to HCl alone (N/10 and N/20) for 30-60 minutes succeeded by Na_2CO_3 0.25%, for as long as 78 minutes failed to induce digestion (Series 14, 15, 16).

E. In the final series (No. 17) frogs exposed to trypsin for more than 2 hours were undigested (confirming Bernard, Dragstedt and others).

Exposure of the legs of living pithed frogs to artificial stomach juice of a physiological concentration of HCl (N/10 and N/20) for such a short time that no digestion could occur, prepares these tissues for digestion by trypsin. This is due partly to a possible destructive effect of HCl *per se,* and partly to the presence of pepsin, as demonstrated by shorter tryptic digestion times when pepsin HCl was employed than when HCl alone was used.

Repeated alternating exposures with short periods of pepsin-HCl and varying periods of trypsin result in more rapid digestion than where the same periods of time are utilized for a single exposure to pepsin-HCl followed by trypsin.

These findings may offer an explanation for the genesis of duodenal and gastro-jejunal ulcer, since these tissues are exposed to successive contacts with acid gastric and alkaline duodenal secretions. This hypothesis must include, however, the existence of an unknown factor which renders the tissues susceptible to digestion. Conditions influencing digestion of living pithed frogs by pepsin-HCl have been discussed in a previous paper.[10]

Summary. The legs of living pithed frogs immersed for a short period of time in HCl or pepsin-HCl without any visible digestion resulting therefrom, can then be digested by trypsin. Primary exposure of the frogs' legs to pepsin-HCl induces a shorter subsequent tryptic digestion time than primary exposure to HCl alone. Therefore, "peptonisation"[11] must play an important rôle in the preparation of these tissues for tryptic digestion. The above experiments demonstrate that tryptic digestion of living tissues can take place under appropriate conditions.

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[11] Kestner, O., *Chemie der Eiweisskoerper,* 1925, page 91.

8789 C

In Vitro Hydrolysis of Fats by Lipase and Bile Salts.

JOHN E. DAVIS. (Introduced by H. B. Haag.)

From the Department of Physiology and Pharmacology, Medical College of Virginia, Richmond, and Department of Physiology, University of Chicago.

The chemical literature apparently reveals no methods for the *in vitro* hydrolysis of animal fats by lipase and bile salts for the special purpose of quantitatively measuring their digestibility. Since it is sometimes desirable to test the relative digestibility of fats which are either untreated or have been treated with a preservative, it occurs to me that an artificial digestion test of this kind might prove useful. I have devised such a test in which I attempt to simulate body conditions of temperature, fat emulsification, gut motility, H ion reaction, and enzyme action. The test was devised for the digestion of lard, although butter and tristearine have also been used as substrate in a few tests.

Method. To 30 gm. lard in a 125 cc. Erlenmeyer flask, add 20 cc. of 0.5% aqueous bile salt solution. Incubate at body temperature for 20 minutes and shake flask to form emulsion. Add enzyme (0.5 gm. Wilson's lipase is about optimum). Place flasks in a wheel, which will rotate 10 times per minute, within an incubator kept at 37°C. Allow fat to digest in this manner for 3, 6, 12 or optional number of hours. At conclusion of digestion period, add 80 cc. benzene to the flasks, set them back in incubator and rotate for 20 minutes more. Remove flasks and place them in ice box for 20 minutes. Pipette off 10 cc. of supernatant benzene, add 3 drops 1% phenolphthalein solution, and titrate with 0.1 N alcoholic sodium hydroxide to a faint pink color. Compute amount of base required to neutralize the fatty acids in the whole benzene phase. A correction of 0.2 cc. should be subtracted, as representing the base required to titrate the benzene phase after a control test in which the substrate is excluded. The resulting acid number is a substantial index to the extent of the hydrolysis which has occurred.

The quantity of substrate to be tested may be reduced (*e. g.*, to

5 or 10 gm.) provided the quantity of bile salt solution and lipase are reduced proportionally.

Calculation:

$$D = \frac{N}{10} \times \frac{M}{1{,}000} \times \frac{f}{f.a.} \times \frac{1}{W} \times 100$$

Where: D = percentage of the fat which has been hydrolized.

N = cc. of 0.1 N NaOH used to titrate whole benzene phase.

M = average molecular weight of the constituent fatty acids in the fat.

$\frac{f}{f.a.}$ = ratio, weight of fat molecule to weight of acids contained therein.

W = weight neutral fat in sample before hydrolysis.

I used a commercial bile salt compound (Wilson) as emulsifying agent. Various concentrations in aqueous solution were tried and a 0.5% solution of the compound proved most effective. The optimum ratio of fat to water for the formation of a stable emulsion was determined by visual inspection and by hydrolysis tests (Table I). The emulsion selected as best promoting hydrolysis is one formed by 3 parts of fat and 2 parts of water; and is an emulsion of water-in-fat.

TABLE I.
Digestibility of Lard in Various Emulsions by a Constant Weight of Lipase (0.5 gm. for 30 gm. lard).

Emulsion gm. lard : cc. 0.5% bile salts in H_2O	% Digestion in 12 hr.
3:2	21.2
1:1	18.9
1:2	12.8
1:4	12.1
1:9	11.8

0.5 gm. of lipase (Wilson) is employed when 30 gm. of lard is to be tested. The initial pH of the bile salt solution before hydrolysis is about 7.3. Its alkali content is not sufficient to saponify an appreciable quantity of fatty acid. Twenty cc. of the 0.5% bile salts solution containing 3 drops of phenolphthalein becomes pink with the addition of 0.05 cc. of 0.1 N sodium hydroxide. The same amount of base is sufficient to titrate to a pink color 20 cc. of our distilled water.

Fig. 1 shows the relationship of time to the hydrolysis of lard. After 24 hours of digestion, an equilibrium point, apparently, has been reached. Dietz[1] showed in an amyl butyrate-water-lipase sys-

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[1] Dietz, W., *Z. physiol. Chem.*, 1907, **52**, 279.

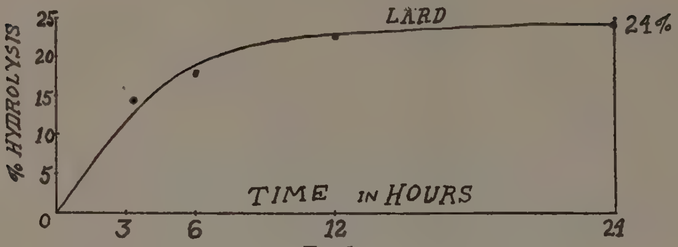

Fig. 1.

tem that a chemical equilibrium comes to exist in which the reverse reaction of ester formation proceeds at exactly the same rate as the hydrolysis. Lipase reactions *in vitro* are probably always reversible.

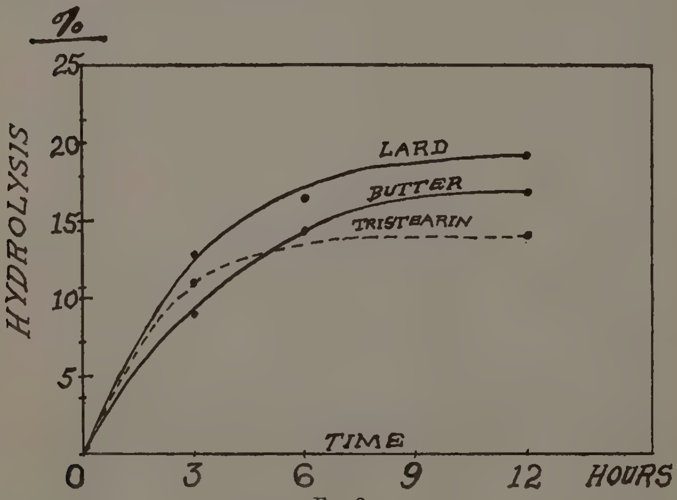

Fig. 2.

Fig. II shows the results of hydrolysis of lard, clarified butter, and tristearine over periods of 3, 6, and 12 hours. Stearine will not form a stable emulsion with the quantity of water specified for lard in this test.

According to Mathews,[2] tripalmitin and tristearine constitute the bulk of the fat of mammals. I have therefore used the figure 270 as the average molecular weight of the fatty acids contained in lard. For the average molecular weight of the fatty acids of butter

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[2] Mathews, A. P., *Physiological Chemistry*, 1925, 4th Ed., 70, Wm. Wood & Co.

I have used the figure 238, which is based upon an analysis by Dean and Hilditch.[3]

Altogether, I have performed about 200 digestions of various durations on lard. In any one digestion test, the percentages of hydrolysis in the separate samples do not differ by more than 0.5% and are usually much closer.

Bile salts alone will not hydrolyse fats at all, but that they are a definite aid to lipase action is shown in a 6-hour digestion test in which lipase alone produced 14.5% hydrolysis of lard, whereas lipase with bile salts produced 16.1% hydrolysis.

Summary. A method is presented for the *in vitro* hydrolysis of fats by bile salts and lipase. In this method an attempt is made to simulate body conditions of temperature, enzyme action, gut motility, and emulsification. The hydrolysis reaches an equilibrium point after about 24 hours, when about 24% of the fat has been broken down.

8790 C

Insulin Absorption from Application to the Skin.

RALPH H. MAJOR.

From the Department of Internal Medicine, University of Kansas School of Medicine, Kansas City, Kansas.

Soon after the discovery of insulin there were several reports in the literature of attempts to introduce insulin into the blood stream by methods other than those of intravenous and subcutaneous administration. Telfer[1] reported that insulin in an inunction when rubbed on the skin of rabbits produced a fall in blood sugar. Harrison[2] repeated this work and found that the inunction of insulin under these conditions had no effect even when introduced in very large amounts. More recently Hermann[3] and Hermann and Kassowitz[4] have reported a marked fall of blood sugar in both animals and patients following the application of an insulin salve on the skin.

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[3] Dean, H. K., and Hilditch, T. P., *Biochem. J.*, 1933, **27**, 889.

[1] Telfer, S. V., *Brit. M. J.*, 1923, **1**, 715.

[2] Harrison, G. A., *Quart. J. Med.*, 1926-27, **20**, 187.

[3] Hermann, Siegwart, *Arch. f. exp. Path. u. Pharm.*, 1935, **179**, 529.

[4] Hermann, Siegwart, und Kassowitz, Heinz, *Arch. f. exp. Path. u. Pharm.*, 1935, **179**, 524.

TABLE I.

Date	Rabbit No.	Time	Blood Sugar, mg.
4/7/36	1	8:40 a.m.	100.5
		9:10 "	25 units insulin.
		10:50 "	68
		12:35 p.m.	90.5
		1:50 "	104.5
4/7/36	2	8:55 a.m.	105
		9:55 "	25 units insulin.
		11:05 "	59
		12:40 p.m.	71
		1:55 "	93
		3:20 "	94
4/9/36	3	9:10 a.m.	127
		9:40 "	25 units insulin.
		11:55 "	69
		1:30 p.m.	88
		2:50 "	121
4/11/36	4	9:20 a.m.	114
		10:00 "	25 units insulin.
		11:45 "	62
		1:15 p.m.	69
		2:45 "	121
4/11/36	5	9:00 a.m.	112
		9:25 "	25 units insulin.
		10:35 "	50.5
		12:10 p.m.	Convulsions.
		12:15 "	25
		12:25 "	10 cc. 50% glucose intravenously. Animal recovered.
		1:55 "	46
4/11/36	6	9:20 a.m.	124.5
		9:40 "	25 units insulin.
		11:55 "	78
		12:30 p.m.	22.5
		1:30 "	In shock.
		1:40 "	24
		1:45 "	10 cc. 50% glucose intravenously. Animal recovered.
4/11/36	7	9:25 a.m.	118
		9:30 "	25 units insulin.
		11:15 "	52
		12:40 p.m.	33
		2:00 "	49
4/21/36	8	9:15 a.m.	124
		10:05 "	25 units insulin.
		11:35 "	65
		1:05 p.m.	51.5
		2:45 "	61
4/21/36	9	9:20 a.m.	128
		10:00 "	25 units insulin.
		11:50 "	52
		1:15 p.m.	16
		1:15 "	Convulsions. 10 cc. 50% glucose intravenously. Animal recovered.
		3:00 "	138

4/24/36	10	9:15 a.m.	118
		9:50 ”	25 units insulin.
		12:05 p.m.	37.5
		1:10 ”	28.5
		1:10 ”	Convulsions. 10 cc. glucose intravenously. Animal recovered.

Details regarding the preparation and composition of this salve are not given.

During the past year in connection with other experiments on insulin absorption[5, 6] we have made numerous attempts to lower the blood sugar by means of dermal applications. Finding that certain glycol compounds aided the absorption of insulin through the mucous membrane, we have employed these and similar compounds as vehicles for the insulin when applied to the skin.

In the course of our experiments we have used a variety of substances as vehicles, including glycerin, triethyanolamin, glycopon, deramin, polycol, propylene glycol, diethylene glycol, glycol laurate, pinacol, di-nitro-benzine, triethylene glycol, decamethylene glycol, and diethylene glycol monoethyl ether. All of these substances are well known chemical compounds except glycopon, polycol and deramin, which are proprietary preparations containing glycols. In addition to these substances salves made of lecithin with methyl salicylate, cantharides, guaicol and menthol were employed. Frankly negative or doubtful results were obtained with all of these combinations with the exception of a mixture of pinacol and insulin and diethylene glycol monoethyl ether and insulin. The results obtained with the latter substance were apparently more striking than those obtained with pinacol.

Recently we have adopted a procedure which on application to the skin of a rabbit has shown an insulin effect. The animal was not fed for 24 hours before the test and the skin of the abdomen having been shaved was rubbed for a few minutes with 3 to 4 drops of glycerin. Ten to 15 minutes later the insulin in diethylene glycol monoethyl ether was applied. This solution was made by mixing one part of an insulin solution with 3 parts of diethylene glycol monoethyl ether. The insulin solution was prepared by dissolving powdered insulin in N/10 HCl. The solution of insulin and diethylene glycol monoethyl ether employed in the tests contained 25 units per 0.1 cc. After application, the solution was rubbed in gently with a glass rod, great care being taken to cause no abrasions

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[5] Major, Ralph H., *J. Lab. and Clin. Med.*, 1935, **21**, 278.

[6] Major, Ralph H., *Am. J. M. Sc.* (in press).

of the skin. A few typical protocols of such experiments on rabbits are given in Table I.

In the course of repeated experiments on the same animals, some rabbits apparently became refractory after the insulin had been applied to the skin of the abdomen 3 or 4 times. It was found, however, that such animals were not refractory if the hair was shaved off the back or the thigh and the insulin applied on this fresh area.

The above observations on normal rabbits indicate that under the proper conditions insulin may be absorbed through the skin and produce a definite and marked fall in blood sugar.

8791 C

Capillary Fragility and Vitamin C.

GEORGE K. ANDERSON,* ESTELLE E. HAWLEY† AND DORAN J. STEPHENS. (Introduced by S. W. Clausen.)

From the Departments of Pediatrics and Medicine, University of Rochester Medical School, Rochester, New York.

It has recently been suggested that tests of capillary resistance may be used as an index of the presence of mild states of vitamin C deficiency or subclinical scurvy.[1-4] Attempts to correlate the capillary resistance with other laboratory and clinical manifestations of scurvy have resulted in conflicting opinions.[5-13]

Two methods, fundamentally different in principle, have been used to determine capillary strength. Both depend on the production of skin hemorrhages, presumably due to rupture of capillary walls. The Rumpel-Leede test, which has been placed on a quantitative basis by the modifications of Göthlin,[1] depends on increasing the intracapillary pressure to the point where petechiae appear in the skin. The "suction" or "negative pressure" test, on the other hand, measures the amount of partial vacuum, applied to the skin, necessary to produce petechiae. The latter procedure has the advantages of being less time-consuming than the Göthlin method and in permitting repeated observations in the same skin area at frequent intervals. The original apparatus of Hecht has been modified by da Silva-Mello,[14] by Dalldorf,[3] and by Cutter and Johnson.[15] Difficulties in the interpretation of the literature arise from the fact that the results of the two methods are not comparable, and may lead to different impressions of the capillary strength.[16, 17]

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* Supported by the University of Rochester Grant for Fluid Research.

† Aided by a grant from the California Fruit Growers Exchange.

[1] Göthlin, Gustaf F., *Skand. Arch. f. Physiol.*, 1931, **61**, 225.

[2] Göthlin, Gustaf F., *J. Lab. and Clin. Med.*, 1933, **18**, 484.

[3] Dalldorf, Gilbert, *Am. J. Dis. Child.*, 1933, **46**, 797.

[4] Stocking, Ruth E., *Arch. Ped.*, 1933, **50**, 823.

[5] Hess, Alfred, *J. A. M. A.*, 1932, **98**, 1429.

[6] Greene, David, *J. A. M. A.*, 1934, **103**, 4.

[7] Perry, C. B., *Lancet*, 1935, **2**, 426.

[8] Schultzer, P., and Grüs, O., *Acta Med. Scand.*, 1935, **85**, 563.

[9] Abt, A. F., Farmer, C. J., and Epstein, I. M., *J. Ped.*, 1936, **8**, 1.

[10] Wright, I. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 475.

[11] Rinehart, James F., *Ann. Int. Med.*, 1935, **9**, 671.

[12] Harris, L. J., and Ray, S. N., *Lancet*, 1935, **1**, 71.

[13] Molitsch, M., *J. Lab. and Clin. Med.*, 1935, **21**, 43.

The purpose of this study was to determine the range and degree of variation of the capillary resistance in normal individuals and to observe its relation to the intake and urinary excretion of ascorbic acid in normal subjects, and the correlation, if any, between capillary strength and specific disease conditions. It should be emphasized that our observations and conclusions relate only to capillary resistance as measured by the suction method. The apparatus used (designed by Dr. S. W. Clausen) consisted of a mercury manometer to which was connected a suction cup and a syringe, by means of which a partial vacuum could be produced, maintained and measured to within one cm. of mercury. The suction cup had an opening one cm. in diameter, with a flange 8 mm. in width, by which the cup could be sealed to the skin with vaseline. The capillary resistance was taken as the least amount of negative pressure (vacuum) in cm. of Hg. applied to the skin for one minute, necessary to produce macroscopic intracutaneous bleeding. In taking the readings, successive, adjacent areas of the skin were taken at intervals of one cm. of Hg., until the lowest level was found at which a single petechial hemorrhage, large enough to be identified by the naked eye, appeared. All readings were made under artificial light—a desk lamp.

A group of 100 normal individuals (75 men, 25 women) between the ages of 15 and 50 were tested. Subjects were, for the most part, physicians, medical students, nurses and other members of the hospital staff. All were in good health. In each instance, inquiry as to the intake of antiscorbutic foods was made and recorded and the capillary resistance determined. Repeated readings at intervals of several days to several months were made in many of the subjects. It has been shown by Weimer,[16] and confirmed by us, that readings obtained by the suction method vary widely in different areas of the skin surface. The majority of our observations have been made on the inner surface of the upper arm, as this area was found to exhibit the most constant results. In a number of instances, readings were taken on the outer surface of the upper arm as well in order that our observations might be compared with those of others who have used this area.

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[14] da Silva-Mello, *München Med. Wchnsch.*, 1929, **41**, 1717.

[15] Cutter, I. S., and Johnson, C. A., *J. A. M. A.*, 1935, **105**, 505.

[16] Weimer, P., *Z. f. d. ges. exp. med.*, 1931, **78**, 229.

[17] Anderson, G. K., unpublished observations.

TABLE I.
Capillary Resistance of Normal Men and Women.

	No. individuals	No. observations	Mean Capillary Resistance mm. Hg.	Standard Deviation	Coefficient of variation
Inner Arm	100	338	13.18 ± 0.141	± 3.83 ± 0.0994	29.2%
Outer Arm	66	245	19.82 ± 0.256	± 5.97 ± 0.165	30.2%

Table I presents the results of a statistical analysis of the observations in normal men and women. Frequency distribution curves of determinations made on the inner and outer surfaces of the upper arm are shown in Fig. 1. A high degree of variation is apparent in both groups. The coefficient of variation of approximately 30% is higher than the majority of anatomic and physiologic characteristics.[18] Further analysis of the results showed no significant effect of age, sex, coloring (blond or brunette), or of season (fall and spring). Although the mean values for the outer arm were significantly higher than those for the inner arm, the spread between the readings taken in both areas at the same time was quite variable. In a number of instances, the capillary resistance determined on the outer arm was only a centimeter or two greater than that determined on the inner arm at the same time. On a few occasions the capillary resistance was lower on the outer arm than on the inner arm.

A second study was made. One hundred patients in the hospital wards were picked at random. Here, again, the values obtained were without significance either individually or when grouped according to disease condition—the range was similar to the normal series.

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[18] Pearl, R., *Medical Biometry and Statistics,* 1923, W. B. Saunders & Co., p. 276.

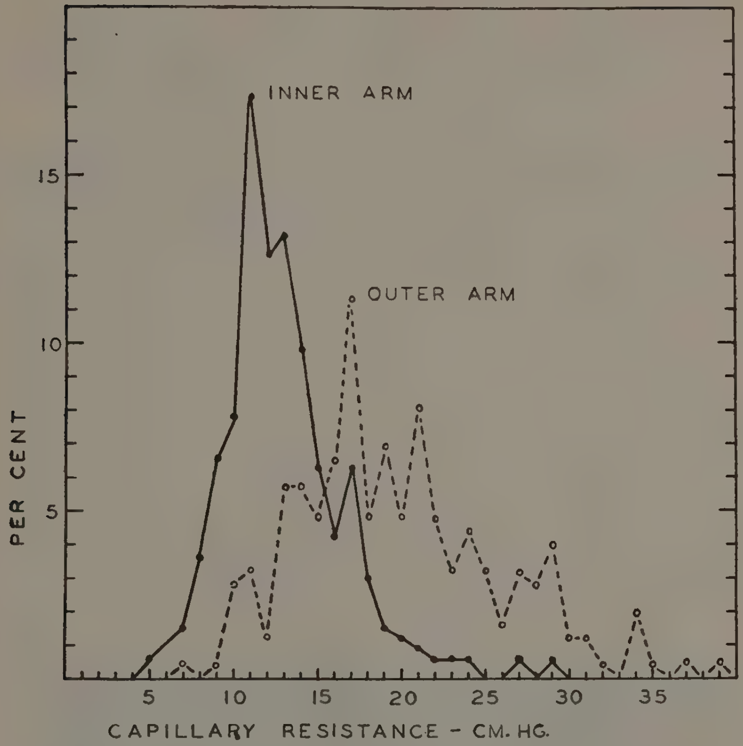

FIG. 1.

Frequency distribution curves of capillary resistance in normal individuals.

Daily observations of the capillary resistance were made in the normal individuals in whom the urinary excretion of ascorbic acid was determined during varying levels of vitamin C intake. The details of this experiment in relation to vitamin C excretion have been reported elsewhere.[17] During a preliminary period of 6 days a diet low in vitamin C was administered. This was followed by the administration of large amounts of vitamin C in the form of 400 cc. of orange juice daily. A delay of from 2 to 9 days in the excretion of the test dose was interpreted as evidence that a mild degree of vitamin C depletion had been induced in each of the 12 subjects. The average values of capillary resistance, summarized in Table II, showed a slight decrease during the period of low vitamin C intake, due to marked changes in a few individuals. The significance of this observation is minimized by the failure of the capillary resistance to increase when large amounts of orange juice were given and evidences of vitamin C saturation appeared. The individual curves of capillary resistance, in some of which there were marked variations, showed no relation to the intake or excretion of vitamin C.

TABLE II.

The Effect of Variations in Vitamin C Intake on the Average Values of Capillary Resistance and Urinary Excretion of Ascorbic Acid in 12 Subjects.

Days	Orange Juice, cc.	Urinary Excretion Ascorbic Acid, mg. per 24 hours	Capillary Resistance, cm. Hg.
1	—	17	17.2
2	—	15	16.0
3	—	16	14.3
4	—	17	13.6
5	—	18	14.2
6	—	12	13.8
7	400	14	12.8
8	400	22	11.0
9	400	69	12.2
10	400	104	13.8
11	400	113	13.6
12	400	120	10.6
13	400	126	12.0
14	400	148	13.8
15	400	133	11.6
16	200	93	11.4
17	200	89	11.6
18	200	83	12.6
19	200	58	12.6
20	200	47	13.4
21	200	49	14.0
22	200	73	14.6
23	200	67	14.0

It is of interest that in an independent, as yet unpublished, study of capillary resistance determined by a method similar to that here described, the threshold level and the range of variation agreed closely with the values observed by us.[20]

Summary. 1. Determinations of capillary resistance to negative pressure were made in 100 normal individuals. 2. Attention is called to the high degree of variation in values for the capillary resistance in normal subjects. 3. Variations in the vitamin C intake of normal individuals, under the conditions of these experiments, had no significant effect on the capillary resistance.

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[19] Hawley, E. E., Stephens, D. J., and Anderson, G. K., *J. Nutrition*, 1936, **11**, 135.

[20] Hauck, Hazel, personal communication, Cornell University, Ithaca, New York.

8792 C

Modification of the Electromyogram of the Simple Reaction by Cerebellar Lesions.

S. R. HATHAWAY AND J. C. MCKINLEY.

From the Division of Nervous and Mental Diseases, University of Minnesota Medical School, Minneapolis.

When simple quick voluntary movements are studied by a combination of action potential and gross movement technics, the contraction of the muscles, and the gross movements occur in a fairly definite sequence. Though finer details vary widely among normals, there are fairly constant gross characteristics.[1] If antagonistic striated muscles are recorded, it will be seen that they will both show potentials within an average time of about 9σ from one another. The contraction potentials of the muscle which will shorten in the response occur slightly ahead of its antagonist. The acting muscle will also contract more strongly than the antagonistic muscle though this difference is not always easily observable on action potential records. At a period of about 60 to 100σ after the appearance of the initial contraction in the muscle, the gross movement begins. (Fig. 1.) These statements are not in accord with the current concepts of the lengthening reaction in antagonistic muscles, but they do substantiate the results of Tilney and Pike[2] who studied this question mainly from the standpoint of muscle volume changes

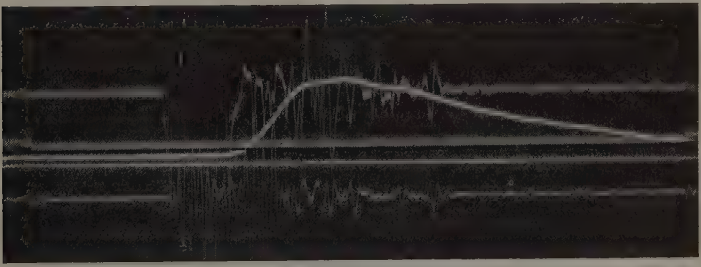

FIG. 1.

Simple extension of the left elbow in a normal. From above downward: Triceps lead, signal line showing stimulus point, gross movement record, 2σ time line, and biceps lead at the bottom.

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[1] Hathaway, S. R., and Sisson, E. D., *J. Exp. Psycho.* (in press).

[2] Tilney, F., and Pike, F. H., *Arch. Neur. and Psychiat.*, 1925, **13**, 289.

in voluntary movement. Weisenburg[3] has said, "If there is a lesion in any part of the cerebellar apparatus there is necessarily produced a disturbance or destruction of the association between agonists and antagonists, and the resulting movement must necessarily be incoordinate or asynergic."

Since we had studied the coordination of simple extension or flexion by the paired action of the triceps and biceps brachii, and to a lesser degree the quadriceps femoris and hamstring group, it seemed advisable to study this response in cases with cerebellar lesions.

The subject was placed in a comfortable position with the forearm supported so that it could rotate freely in a horizontal plane about the elbow. He was instructed to extend (or flex) the forearm as quickly as he could after the appearance of a light which was used for a stimulus. The same technic was used for the extension of the lower leg at the knee joint except that in this case no support was used, the lower leg being simply kicked forward. These movements were practiced before the pictures were actually taken. The records show the action potentials in the muscles involved, the gross movement, the point of appearance of the signal, and a time line for reading the time differences involved. The slope of the gross movement line is an approximate value for the force of the movement.

We also made several records of the cerebellar rebound phenomenon. The recording technics were essentially the same in this case, the subject being instructed to hold with considerable force against

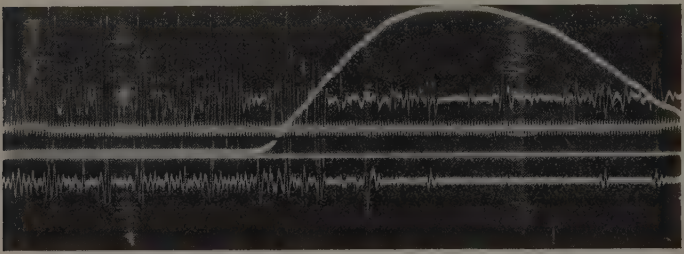

FIG. 2.

Cerebellar rebound in a normal. First pull against triceps. From above downward: Triceps lead, 5σ time line, gross movement record, and biceps lead at bottom.

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[3] Proc. of the Assn. for Research in Nervous and Ment. Disease. VI. The Cerebellum, 1929, Baltimore.

a constant pull on the forearm. This pull was released with the appearance of the signal, allowing the subject's forearm to swing suddenly outward or inward as the case might be. (Fig. 2.)

We are reporting at this time the findings in two cases of cerebellar involvement. The first was a fairly advanced case of Marie's cerebellar ataxia in a man, age 35. He is at present unable to walk without support. The cerebellar symptoms are marked, and earlier in our observations were the only symptoms and signs from the nervous system. During the past year, there have been superimposed on the original syndrome both increasing deep reflexes and decreasing deep sensibility in the extremities. This subject's records show several definite abnormalities. The most obvious difference from the normal occurs in the antagonistic muscle which, in contradistinction to the normal, is but little involved in the reaction. This subject, then, shows in these movements a practically unopposed contraction of the acting muscle (Fig. 3). This is true both in the arm and in the thigh. The reaction time is much longer than average and the gross movement tends to be somewhat wavering; the patient was nevertheless very cooperative and obviously doing his best.

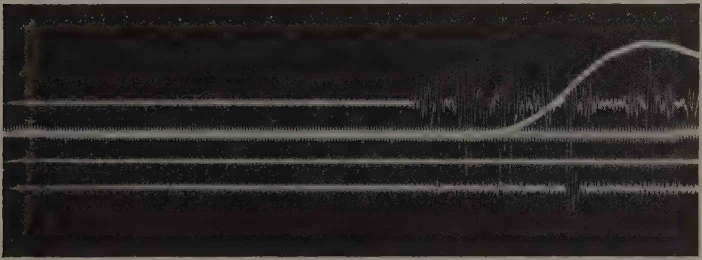

FIG. 3.

Simple extension of the left elbow in a case of Marie's cerebellar ataxia. From above downward: Triceps lead, superimposed gross movement and 5σ time lines with break in time line marking stimulus, and biceps lead at bottom.

Records of the cerebellar rebound show the antagonistic muscle coming in strongly as a reflex response, possibly myotatic, to the extension of that muscle which occurs when the arm is released. This action of the antagonist not only suddenly stops the outward movement of the released forearm, but brings it back so rapidly that the opposite type of contraction occurs in the other muscle producing a second extension and the typical rebound phenomena. Normal rec-

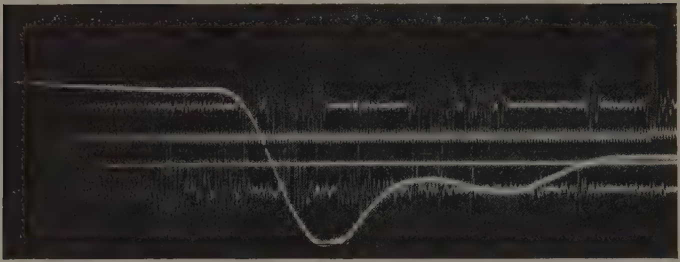

FIG. 4.
Cerebellar rebound in the left arm of a case of Marie's cerebellar ataxia. First pull against biceps. From above downward: Gross movement record, triceps lead, 5σ time line, and biceps lead at bottom.

ords of this same reaction do not show this interplay of antagonistic muscles (Figs. 2 and 4).

In view of the definite findings in this case, it seemed advisable to find a case in which the loss of cerebellar tissue would be much less marked and in which there would be no other demonstrable sensory and motor tract signs.

We selected from the clinic a 12-year-old girl, who, about 2 years previously had had an astrocytoma removed from the left lobe of the cerebellum. She was originally markedly ataxic but at present is neurologically negative. Records in this case (Fig. 5) were much more nearly normal. Reactions were within normal reaction time and in general appeared to the observer to be normal. When the records are closely examined, however, the antagonists are found to be involved in the reaction much less than the normal and the

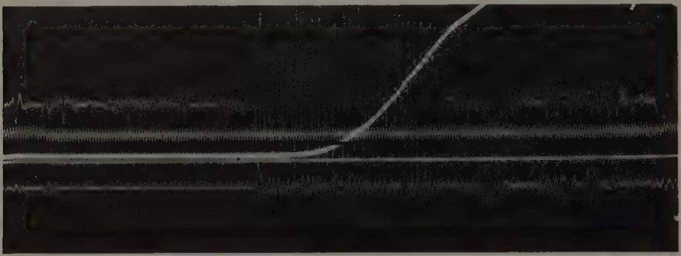

FIG. 5.
Simple extension of left elbow in a case with a small cerebellar lesion. From above downward: Triceps lead, 5σ time line with break showing stimulus point, gross movement record, and biceps lead at bottom. The preliminary tone in the triceps should be disregarded.

time between the appearance of contraction in the two muscles is usually about twice the normal time. This is particularly true in the left arm, which was originally the more ataxic. The records of cerebellar rebound were apparently normal.

As a check upon the possible sources of these disturbances especially regarding our first case, we obtained records from a moderately advanced uncomplicated tabetic patient who had complete loss of tendon pain, a much reduced muscle pain, and absent patellar reflexes. On this subject we obtained records from the left arm and leg. These records were not different from normal in any of the above points. This seems to eliminate the possibility that proprioceptors are determinants of activity of the antagonist in a simple reaction.

Though 2 cases of cerebellar disease do not constitute a satisfactory series, it is striking that they deviate in a similar way from the normal in recordings of simple reactions. Thus, instead of the nearly simultaneous contraction of agonist and antagonist which we have observed in normals, the cerebellar cases show a suppression of potentials in the antagonist. A tendency to increased reaction time is also notable. A generalization from these observations as to their applicability for the diagnosis of cerebellar involvement must obviously await the accumulation of more data.

8793 P

Rapid Procedure for the Quantitative Estimation of Antitrypsin.

KENNETH L. BURDON AND CHARLES LAFFERTY.

From the Department of Pathology and Bacteriology, Louisiana State University Medical Center, New Orleans, Louisiana.

Gates,[1] Gilman and Cowgill,[2] and Pickford and Dorris,[3] have found that photographic film furnishes a useful substratum for the titration of proteolytic enzymes, since the gelatin layer, which is readily digested by pepsin or trypsin, is sufficiently uniform in thickness and consistency to permit quantitative tests. The release of the silver from an exposed (opaque) film, through the progressive proteolysis of the gelatin, causes a proportionate decrease in density, and finally, when digestion is complete, the film becomes entirely transparent.

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[1] Gates, F. L., PROC. SOC. EXP. BIOL. AND MED., 1926-27, **24**, 936.

[2] Gilman, A., and Cowgill, G. R., *J. Biol. Chem.*, 1930, **88**, 743.

[3] Pickford, G. E., and Dorris, F., *Science*, 1934, **80**, 317.

We have utilized this principle in devising a new technique for the quantitative titration of the antitryptic power of serum or other fluids. At the same time, we have modified and simplified the procedures followed by the authors mentioned. We have avoided the use of the colorimeter for estimating changes in the density of the film, as advocated by Gilman and Cowgill, and instead take for the end-point the film showing complete transparency to the naked eye, thus measuring digestion of the gelatin only, without reference to an arbitrary standard of initial density. After determining by experiment the influence of various factors on the reaction, we are able to outline a definite procedure, which permits the exact reproduction of results at will, and the expression of the findings in simple figures. Further, we have provided for the preservation of the films themselves as a permanent record.

The following is the procedure now recommended.

Eastman Process film is fully exposed to light, and developed for 5 minutes in D-72 developer. It is then cleared and fixed in freshly prepared hypo solution, containing the usual proportion of F1—a hardener, for 15 minutes. After thorough washing and drying, the film is cut into discs ¼ inch in diameter with a hand punch.

A stock 2.0% solution of Fairchild's trypsin is made in a borax-boric acid, salt, buffer solution, pH 7.3, prepared according to the directions of Palitzsch, as given by Clark.[4] One gm. of the powdered trypsin is stirred into 50 cc. of the buffer solution, and the mixture allowed to stand at room temperature, with frequent shaking of the flask, for 15 minutes. It is then filtered into a sterile, screw-top bottle, and 0.1 cc. of a 1:1000 aqueous solution of merthiolate is added. Kept in the refrigerator, this stock solution loses potency gradually, but remains sufficiently active for at least 10 days.

Human serum to be tested for antitryptic power is diluted with 4 parts of the buffer solution just before use.

A series of dilutions of the stock trypsin solution is prepared by pipetting into test tubes amounts of this solution, beginning with 0.1 cc., and increasing each time by 0.1 cc., and then adding sufficient buffer solution to bring the total volume in each tube to 2.0 cc. A range of 8 or 10 dilutions is usually enough to give convenient end-points.

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[4] Clark, W. M., *The Determination of Hydrogen Ions*, Williams and Wilkins Co., Baltimore, 1923, pp. 115, 117.

Using separate, cotton-plugged pipettes, equal quantities (0.3 cc.) of each dilution and of buffer solution (in titration of the trypsin alone), or of diluted serum (in titration of antitrypsin), are added to a 2nd series of small test tubes, and the fluids thoroughly mixed. A control tube is prepared, containing 0.6 cc. of buffer solution only.

These tubes are now placed in a water bath at 40°C. for 10 minutes, solely for the purpose of warming the test fluids. The contents of each tube are then poured quickly into separate concavities of a Coors glazed porcelain plate, previously warmed for at least 15 minutes in a 40°C. incubator. A single film disc, handled with forceps, is now dropped, gelatin side down, upon the surface of each mixture. The plate is returned at once to the 40°C. incubator, the atmosphere of which is kept saturated with moisture, and digestion is allowed to proceed for 15 minutes.

The plate is then removed, and, as rapidly as possible, each film disc is picked up with forceps, rinsed briefly in 4% formalin (to stop the reaction and fix the film), and deposited, gelatin side up, upon filter paper to drain. The discs are now mounted upon a strip of transparent, gummed cellulose tape stretched across an opening prepared in a 4 by 6 inch card. As soon as the films have dried, a 2nd strip of the tape is placed over them, sealing them between the gummed surfaces. The card, which may now be freely handled, bears all data necessary to describe the experiment and may conveniently be filed as a permanent record.

When viewed against the light the first of the film discs in the series to be fully cleared is apparent to the naked eye. This is taken as the end-point, and the titer of tryptic activity is expressed directly in terms of milligrams of trypsin per cc. in this mixture, containing the lowest concentration of trypsin, which has brought about complete digestion. Thus, a fresh 2.0% trypsin solution will almost invariably have a titer of 1.0, *i. e.,* complete digestion will first occur in the mixture made by diluting 0.2 cc. of this solution up to 2.0 cc. and further diluting, for the test, with an equal volume of buffer solution, so that the final concentration of trypsin per cc. in this mixture is 1.0 mg.

The difference between the titer in the presence of a diluted serum and the titer in the absence of serum represents the antitryptic power of that serum. This figure, multiplied by the serum dilution, is assumed to express the *antitryptic index* of the undiluted serum.

In preliminary titrations, the sera from 50 normal women showed a mean antitryptic index of approximately 7.5, whereas the mean value for the sera of 15 pregnant women was found to be approximately 10.0. This is in accord with the findings of others, who have observed a rise in antitrypsin during pregnancy.[5]

8794 P

Pregnancy in Cholesterol Fed Rats.

LOIS STEWART GODFREY* AND RUTH OKEY.
From the Laboratory of Household Science, University of California, Berkeley.

The disturbances in blood cholesterol level known to be associated with pregnancy, and the prevailing impression that pregnancy may increase the incidence of diseases associated with abnormal cholesterol deposition have indicated the desirability for study of the influence of cholesterol feeding during pregnancy.

There is strikingly little record of such a study in the literature. Schönheimer[1] reported that pregnant animals were more subject to the deposition of anisotropic fat than non-pregnant ones. He reports feeding cholesterol to a rabbit which became very sick 14 days after mating and showed resorption of 3 fetuses with one fetus dead at term and abnormal deposition of anisotropic fat in the placenta.

We have used rats for the present study. These were placed at weaning on a diet made up of 20 parts raw casein, 4 parts Osborne and Mendel salts, 4 parts agar, 15 parts Crisco, and 57 parts starch, with one part cholesterol dissolved in Crisco and incorporated in the diet. Vitamin supplements were given separately 3 times a week as yeast, tiki-tiki, or yeast extract for B, and tuna or sea bass liver oil standardized in this laboratory and diluted with corn oil for A and D. With tiki-tiki, raw casein supplied G. After successful mating was demonstrated by the finding of sperm, the protein in the diet was increased to 26% and the starch decreased to 51% and the vitamin B increased 2 to 4 times (in different groups). This diet was modeled on that shown by Morgan and Simson[2] in this laboratory to be adequate to meet the food requirements of the rat during pregnancy.

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[5] Flexner, L. B., Berkson, J., Winters, H., and Wolman, I., PROC. SOC. EXP. BIOL. AND MED., 1928-29, **26**, 592.

* Deceased, November 16, 1935.

[1] Schönheimer, R., *Arch. Path. Anat.* (Virchow), 1924, **249**, 1.

[2] Thesis: Catherine M. Cave Simson with A. F. Morgan, Department of Household Science, University of California, Berkeley, 1934.

Vaginal smears were taken daily after the rats were approximately 90 days of age. In general cycles tended to be lengthened by one to 2 days. They were not altered, nor was the mating performance of the animals changed when, at the suggestion of Mrs. O. H. Emerson, a wheat germ oil preparation of tested potency in amounts several times that necessary to supply the vitamin E requirement for normal pregnancy in the rat was given to half the animals. Some difficulty was experienced in obtaining successful matings with cholesterol-fed animals. There seemed, however, to be no difference, either in fertility or in difficulty of mating when cholesterol-fed males or males on stock diets were used. Likewise, cholesterol-fed males showed no loss of fertility when mated with stock females.

Fifteen first and 5 second litters from cholesterol-fed mothers were carried through to term. Thirteen apparently entirely normal animals were killed just before delivery and nine more from the 14th to the 18th days of pregnancy. Three animals showing resorption were killed during pregnancy. Because of the previous study carried out on the same diet in this laboratory, only about half that number of non-cholesterol-fed animals were included in this first series. These were sacrificed during and just at the end of their pregnancies.

The livers of the cholesterol-fed mothers showed the usual fatty appearance. There was also some gross evidence of fatty infiltration of the placentae. Fetuses tended to be slightly smaller than in control and stock animals killed at the same stages of pregnancy. Birth weights of the young were not obtained because we were afraid that handling the animals would induce cannibalism. Two litters were found dead within a day after birth, and one or 2 animals were lost from 3 other litters. Litter size averaged 8.5 for cholesterol-fed mothers, a number not significantly different from the controls but about 4 less than for the stock colony. Eighteen litters were carried to weaning. Weaning weights of babies from cholesterol-fed mothers averaged 20.5 gm., which was more than 15 gm. lower than Morgan and Simson's for the same basal diet, and over 20 gm. lower than the average from our stock colony for litters cut to this size. These animals, however, grew normally after weaning, the average weights of 30 females kept for an additional 6 months on the cholesterol diet was 193 gm. and the average for 15 males, 305 gm.

It would seem, therefore, that we are justified in concluding that, while cholesterol feeding apparently interferes to some extent with intrauterine growth, we may, nevertheless, carry cholesterol-fed animals through successful pregnancy and lactation studies. Because it is possible to follow cholesterol distribution in tissue, this should represent a useful method for study of the lipid transport.

Tissue analyses from these and later series of pregnant animals will be reported in the future. Mrs. Godfrey's untimely death has necessitated reorganization of our plans for this study and delayed the work to the extent that we have felt that the potential usefulness of her findings has indicated this preliminary report.

8795 P

Isolation of a Water-Soluble Pregnandiol Complex from Human Pregnancy Urine.

ELEANOR M. VENNING AND J. S. L. BROWNE. (Introduced by J. C. Meakins.)

From the McGill University Clinic, Royal Victoria Hospital, Montreal, Canada.

A physiologically inactive solid alcohol was prepared from human pregnancy urine by Marrian[1] in 1929. This substance was identified by Butenandt[2] in 1930 and was called by him pregnandiol. It was insoluble in water and showed a melting point of 233-235°C. (uncorrected). Recently O'Dell and Marrian[3] have obtained evidence for the existence of an acid-hydrolyzable form of pregnandiol.

We have been able to isolate a water-soluble complex of pregnandiol from pregnancy urine. The method of preparation was as follows: Pregnancy urine (9th month) was extracted with butyl alcohol. The extract was evaporated to dryness under reduced pressure. The residue was taken up in N/2 NaOH and re-extracted with butyl alcohol, the butyl alcohol fraction washed twice with water and evaporated to dryness. The residue was dissolved in a minimal amount of water and the substance was precipitated with acetone. This was collected by centrifuging and purified by crystallization from hot water and several times from ethyl alcohol.

The white crystalline substance, so obtained, melts at 268-71°C. (uncorrected) with decomposition and evolution of gas. It crystallizes from alcohol in thin plates. It is soluble in water, less soluble in ethyl alcohol, insoluble in ether. Qualitative tests for nitrogen, sulphur, phosphorus and halogens were negative. It is precipitated from aqueous solution at room temperature by acid, yielding a crystalline compound of acidic nature, which melts at 179-180°C. (uncorrected) and decomposes with gas formation.

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[1] Marrian, G. F., *Biochem. J.*, 1929, **23**, 1090.

[2] Butenandt, A., *Ber. dtsch. chem. Ges.*, 1930, **63**, 659.

[3] O'Dell, A. D., and Marrian, G. F., *Canadian Chemical Convention*, 1936, June 11th.

The material melting at 271°C. was subjected to acid hydrolysis in the autoclave, and a crystalline substance was obtained (insoluble in water but soluble in acetone) melting at 218°C., which on recrystallization from acetone and alcohol melted at 233°C. It showed no depression of mixed melting point with pregnandiol. A solution of the complex showed no reduction of alkaline copper, however, the filtrate after hydrolysis caused marked reduction. Both the filtrate and the original material gave a strongly positive naphthoresorcinol test (Tollens) showing the presence of glucuronic acid.

The carbon-hydrogen analyses for the free acid gave the following values: C, 63.65%, 63.52%; H, 8.75%, 8.77%. The theoretical values for pregnandiol-glucuronic acid ($C_{27}H_{44}O_8H_2O$) are C, 63.1%; H, 8.95%. The values for the acid agree reasonably well with those required for pregnandiol-glucuronic acid. The complex extracted directly from the urine contains sodium and is a monosodium salt of pregnandiol glucuronic acid. The yield of the pregnandiol complex obtained by this method as the sodium salt, from pregnancy urine near term, is at least 40 to 50 mg. per liter, which on hydrolysis would give about 20 to 25 mg. of pregnandiol, a greater amount than has previously been reported.

As pregnandiol may be regarded as the excretion product of corpus luteum hormone and also is the initial product in the synthesis of this hormone, the above relatively easy method of extracting and measuring this substance should be of practical significance.

We are at present measuring the amount of this complex excreted during the various stages of pregnancy.*

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* Carbon-hydrogen analyses were done by Dr. V. Niederl, New York University.

8796 P

Effects of Anatomical Separation of Hypophysis from Hypothalamus in the Dog.*

A. D. KELLER, WILLIAM NOBLE AND J. W. HAMILTON, JR.

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

With a view of elucidating the mechanism concerned in eliciting enhanced food intake following removal of the posterior lobe of the hypophysis and hypothalamic lesions, the whole of the hypophysis was separated from the nerve and blood supply coming to it from the hypothalamus by cutting the stalk with scissors at its junction with the hypothalamus. This was successfully accomplished in 5 dogs, as evidenced by direct observation and by serial section of the tissue, without injury to the pars anterior, pars intermedia, or hypothalamus, and with variable, though slight, involvement of the pars tuberalis. In all animals there occurred a central atrophy of the infundibular process and increased cellularity in its remaining portion. The duration of the experiments ranged from three to five weeks.

A slight increase in food and water consumption occurred temporarily and showed a tendency to persist in instances, while in others there remained no deviation from the normal. There was no significant alteration in blood sugar, rectal temperature or in the general behavior of the animals either directly following operation or subsequently. These results demonstrate that in the dog, as in the monkey,[1] the blood supply from the posterior lobe artery is adequate for the gland as a whole; and also that denervation (hypothalamic) of the pars nervosa (which includes the infundibulum) does not precipitate enhanced food consumption or diabetes insipidus.

In order to harmonize these results in the dog with the interpretations of the Richter and Ranson schools relative to the cause of diabetes insipidus, one would have to assume either (1) that the ventral portion of the hypothalamus serves as a source of the antidiuretic principle as well as the pars nervosa, or (2) that our procedure in some way paralyzes a possible hypophyseal diuretic mechanism. It seems certain that the latter is not the case since

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* This work was aided by a grant from the Rockefeller Foundation.

[1] Mahoney, W., and Sheehan, D., *Brain*, 1936, **59**, 61.

marked transitory diabetes insipidus (700 cc. of water per kg. body weight) occurred in four dogs in which following separation of the gland its proximal surface was cauterized. In a few days, the water intake returned to normal or only a trace of an increased consumption persisted. In one instance following cautery, typical diabetes insipidus appeared subsequent to the 10th day; thus suggesting that the essential mechanism responsible for diabetes insipidus both of the temporary and of the permanent type is inherent in the hypophysis. That the ventral portion of the hypothalamus is not involved as outlined above is indicated not only by numerous protocols from our earlier cat and dog series but also by further experiments for verification now near completion.

That the hypophysis is essential for the diabetes insipidus which follows hypothalamic lesions is also evidenced because hypophysectomy, on the 49th day after the hypothalamic lesion, eliminated abruptly a persistent marked diabetes insipidus (from 700 cc. water per kg. of body weight to normal—100 cc.±). This result might of course be due to an indirect effect on metabolism rather than to the removal of the specific mechanism causing the disturbance.

8797 C

Effect of Viosterol on the Parathyroids and Kidneys of Rats.*

E. V. COWDRY AND L. E. RECTOR.

From the Anatomical Laboratory, Washington University, St. Louis.

Alterations unlike those hitherto reported as following the administration of viosterol have been recently described in monkeys *(Macacus rhesus)*.[1] The parathyroids of some remained about the same, but those of others showed a marked decrease in basophilic cytoplasmic material, and the possibility was mentioned that this material might constitute a parathyroid secretion antecedent. In the kidneys, the incidence of intranuclear inclusions increased from 18.7 to 75% of individual monkeys. This was interpreted as due, either to the activation of a virus already present in very small amounts, or to the direct effect of the treatment. It was not possible to correlate the degree of loss of parathyroid basophilic ma-

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* Aided by a grant from the Josiah Macy, Jr., Foundation.

[1] Cowdry, E. V., and Scott, G. H., *Arch. Path.* (in press).

terial, or the extent of formation of the nuclear inclusions in the kidney with the size of the dosage.

White rats were selected for further experiments because Rector[2] found that in our laboratory the kidneys of 50 were apparently without nuclear inclusions. This indicated that, in the particular stock of rats examined, the likelihood of a virus being present in the kidneys was remote as contrasted with the probability that such a virus existed in the kidneys of the monkeys as evidenced by the occasional occurrence of nuclear inclusions in the absence of treatment with viosterol. Realizing the importance of accurate controls, especially in measuring the parathyroid change, 52 rats were selected from our colony on the basis of sex and weight so that 26 of them (sexes equally divided) were comparable individually with the remainder as to sex and weight measured to the gram. Each rat of the first group of 26 was given 0.2 cc. of the strong preparation of viosterol manufactured by Mead, Johnson Co. (containing 1,000,000 international units of vitamin D per cc.)† every other day. But soon the dosage was reduced owing to its evident toxicity and, in the case of some rats, it was stopped altogether. Whenever a rat died its untreated running mate of the same sex, and originally of the same weight, was killed and histological preparations were made of parathyroids, kidneys and aorta. Thirty days after the treatment was commenced, all the surviving animals with their running mates were killed and examined in the same way. The lesions reported in the literature as produced in rats by hypervitaminosis D were observed; but parathyroid and renal alterations, comparable to those mentioned in monkeys, were conspicuous by their absence. No similar parathyroid basophilic cytoplasmic material was found, even in the untreated rats, and no typical nuclear inclusions were observed in either group.

It is evident, therefore, (1) that the normal structure of the parathyroid cells of the rat is quite different from that of the monkey and (2) that dosage with viosterol did not excite nuclear inclusion formation in the kidneys of the rat—a species in which, in our colony, there was no cytological evidence of a virus to begin with.

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[2] Rector, L. E., PROC. SOC. EXP. BIOL. AND MED. (in press).

† Kindly supplied for these experiments by Mead Johnson Co.

8798 P

Experiments on Decalcifying Anticoagulants.

JOHN H. FERGUSON.

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

Reagents used.[1] 1. Prothrombin-free fibrinogen (dog). 2. Howell prothrombin. 3. (Calf) brain cephalin (active in 1:2,000,000 dilution). 4. M/10 $CaCl_2$. 5. M/1 (etc.) potassium oxalate and trisodium citrate. Incubations and tests conducted at 37-38°C.

Controls. An initial series of tests established the lower limits of the calcium optimum in the activation of prothrombin-cephalin mixtures. Incubation of coagulant mixtures for at least 10 minutes prior to addition of fibrinogen ensured thrombin of maximal potency: *e. g.,* 1 cc. of fibrinogen was clotted solidly in a matter of seconds by the following: prothrombin, 0.5 cc. + 0.1% cephalin, 0.1 cc. + M/10 $CaCl_2$, 0.1 cc. In order to validate the significance of the clotting time (C.T.) delay in the accompanying experiments, routine controls were run with and without simple dilution (distilled water or 0.9% NaCl). The control thrombins retained their potency with but slight diminution of activity (? metathrombin formation) over several hours.

Oxalation (or citration) during thrombin formation and aging. A 30-50-fold excess (chemical equivalents) of oxalate (or citrate): added calcium (of the thrombic mixture) entirely prevented the formation of thrombin if the anticoagulant was added during the first 4-5 minutes of incubation. During the next hour the already activated thrombin gave good clots although somewhat delayed by the presence of oxalate (or citrate). Older thrombins yielded progressively slower and poorer clots in contact with the anticoagulant.

Effect of increasing concentrations of anticoagulant on thrombin of fixed (maximal) potency. Fresh (maximally active) thrombin gave correspondingly longer clotting times when added to a series of test fibrinogens containing increasing amounts of oxalate or citrate. There was a minimum below which the C. T. was identical with controls, and a maximum above which the C. T. delay was not significantly extended. Indeed, with very great excess, especially in the citrate experiments (verified repeatedly), a return of C. T. to definitely shorter values was noted. A possible correlation with clinical haemostasis by citrate injections is mooted.

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[1] Ferguson, J. H., *Am. J. Physiol.*, 1936 (in press).

Progressive inactivation of thrombin on incubation with oxalate or citrate. After maximal potency had developed (*e. g.,* 10-15 min. incubation) the thrombin was divided into two parts, mixed respectively with (a) 50-100 equivalents of oxalate or citrate, and (b) an equal volume of simple diluent, and the incubation continued. Tests made at varying intervals ranging from a few seconds to 2 hours invariably demonstrated (1) an *immediate* retarding effect of the anticoagulant, varying in degree according to the concentration of oxalate (or citrate) used and comparable to the findings of the foregoing experiments; and (2) a *progressive* inactivation, resembling the phenomenon described by Loucks and Scott.[2] Whereas controls still clotted in a minute or two (even after 2 hours), the oxalated (or citrated) thrombin could be weakened to give clots only after many minutes or hours or (in a few instances) not at all. The inhibitory phenomenon was also manifest in the slower completion of clotting after its commencement, and certain of the delayed clots were flocculent and incomplete.

Conclusion. It has long been established that thrombin, once formed, is active in the presence of excess of oxalate, etc., this being the classical experiment in support of the view that ionized calcium is not necessary for the clotting process proper ("second phase" of Hammarsten). It is less widely appreciated that clotting under these conditions is definitely slower than normal. Forty years ago Hammarsten[3] failed to inactivate thrombin by oxalation, and Kastl[4] and Eagle[5] have recently confirmed. The divergent findings of Loucks and Scott[2] are supported by the present experiments. Since the data appear to be unequivocal, and since their importance in the elucidation of the calcium factor in coagulation is obvious, it is imperative that further investigations be made with a view to explaining the discrepancies in the literature and elucidating further the mechanism of action of the so-called "decalcifying" anticoagulants.

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[2] Loucks, M. M., and Scott, F. H., *Am. J. Physiol.*, 1930, **91**, 27.

[3] Hammarsten, O., *Hoppe Seylers Ztschr. f. physiol. Chem.*, 1896, **22**, 333; 1899, **28**, 98.

[4] Kastl, O., *Biochem. Ztschr.*, 1934, **274**, 452.

[5] Eagle, H., *J. Gen. Physiol.*, 1935, **18**, 531, 547.

8799 P

Physicochemical Mechanisms in Convulsive Reactivity.*

E. Spiegel and M. Spiegel-Adolf.

From the Department of Experimental Neurology and the Department of Colloid Chemistry, D. J. McCarthy Foundation, Temple University, School of Medicine, Philadelphia.

Since convulsions may be called forth by a great variety of causes, the question presents itself as to whether a common fundamental mechanism exists by which the epileptogenous agents act upon the nerve cells. According to Nernst, Bethe, and others, excitation is due to changes of ion concentration on semi-permeable cell surfaces. These changes in ion concentration are supposed to influence the nerve cells by increasing the cellular permeability (Hoeber, Lillie). One should, therefore, expect that agents which lower the density should also increase the excitability.

In order to study the effect of various epileptogenous agents upon the permeability in living animals (cats, rabbits), a method of measuring the permeability was employed similar to that used on artificial membranes.[1]

The conductivity of the part of the brain under study was determined by using alternating currents of various frequencies. Due to polarization on the cellular surfaces and interfaces the conductivity is the lower, the lower frequencies are used. It can be shown on artificial membranes that the difference in conductivity at a certain high and at a certain low frequency (polarization index Δ†) is increased, if the membranes become more impermeable, and that it is diminished, if the permeability is increased. The measurement of Δ can, therefore, be used in order to study the permeability of the cellular surface films not only *in vitro,* but also *in vivo,* on animals, or on patients during operations. Δ is higher in the grey matter than in the white matter of the cerebral hemispheres, indicating the part played by the nerve cells in these polarization phenomena.

A study of the effects of the most important causes of convulsive disorders upon Δ gave the following results. Anoxemia

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* This work was aided by a grant of the American Association for the Advancement of Science.

[1] Spiegel-Adolf, M., and Spiegel, E., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 139.

† This difference was always expressed in percentage of the conductivity at the lower frequency.

(asphyxia) increases the cellular permeability in the cerebral hemispheres as well as in the subcortical ganglia. Anemia, as produced by ligature of the cerebral arteries, diminishes the conductivity. In the majority of the experiments an initial drop of Δ was also observed; when the anemia is continued for several minutes, the curve of Δ may again rise, probably due to coagulation necrosis of a number of cells. Increase of intracranial pressure also lowers the conductivity; this is associated in the majority of the experiments with a drop of Δ. Increase in the state of hydration (*e. g.*, by intravenous injection of distilled water or by artificial alcalosis) increases the permeability, independently of changes in conductivity. This effect is still observed if an increase in intracranial pressure is prevented by trephining the cranium. While alcalosis is accompanied by increase in permeability, acidosis has only a slight effect upon Δ. Hyperventilation acts in a similar way, as does alcalosis. These effects are reversible. Anesthetics and hypnotics, such as ether, chloroform, dial, have the opposite effect; they increase Δ, indicating an increase in density of the cellular films.

The inference is drawn from these experiments that epileptogenous agents may act upon the nervous system by either of the following mechanisms (or by both): 1st. Production of a change in ion concentration on the surfaces of the nerve cells (as for instance by injection of acids). If the change in concentration reaches the threshold, excitation (convulsion) results.

2nd. Diminution of the density of the cellular surface films (as observed in anoxemia, hydration, alcalosis). The increase in permeability of the cell surface that is an important part of the excitation process is facilitated, and the threshold of the cells for metabolic or other stimuli is lowered, the convulsive reactivity is increased. Thus the problem of convulsive reactivity is only part of the more general problem of relationship between excitability and permeability of cellular surface films.

8800 C

Resistance of Thyroparathyroidectomized Rats to Growth of a Spindle-Cell Sarcoma.

F. A. McJunkin, R. D. Templeton and F. G. Kravec.

From the Departments of Pathology and Physiology, Loyola University School of Medicine.

The published reports on the effect of thyroidectomy on neoplastic growth present divergent opinions. Rohdenburg, Bullock and Johnston[1] in a series of 29 thyroidectomized rats found at 40 days after inoculation with Flexner-Jobling carcinoma 52% of "cures" against only 16% of regressions in the control group. Recently Bischoff and Maxwell[2] inoculated 13 thyroparathyroidectomized rats with rat carcinoma 256. Based on a comparison with 16 inoculated litter-mate controls they observed "no significant difference in percentage of takes, rate of tumor growth, or final mortality". Our tumor, a sarcoma, which arose spontaneously in a rat uterus has been transplanted during the past three years into more than 1000 rats. Successfully inoculated tumors in normal rats have not been seen to regress. This is mentioned to indicate that a spontaneous regression of this tumor, if it does occur, is uncommon. The operation consists of complete ablation of thyroid and parathyroid glands. All rats were from our colony of Wistar Institute albino stock. All individuals of the litters, or groups, were inoculated bilaterally into groin pockets from the same tumor and at the same time.

In Table I where the tabulation is by litters the 4 longest post-thyroidectomy ones (1, 2, 3, 4) are placed first. Of the 18 successful takes in this group 14 completely regressed. This high percentage (77%) of regressions in a group where the shortest post-thyroidectomy period was 112 days is in contrast with 25% of regressions in all successfully inoculated remaining rats with shorter post-operative periods. In both groups it is seen that a first inoculation may fail and a second one succeed. This is true also for normal control animals, and sometimes may result from the accidental introduction of bacteria into the pocket. In the rats with relatively short post-thyroidectomy periods there is an usual tendency for the second inoculation tumors to regress. This may be explained in

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[1] Rohdenburg, G. L., Bullock, F. D., and Johnston, P. J., *Arch. Int. Med.*, 1911, **7**, 491.

[2] Bischoff, F., and Maxwell, L. C., *J. Pharm. and Exp. Therap.*, 1932, **46**, 51.

TABLE I.

Litter or group No.	e = thyro-parathyroid-ectomized rats c = litter-mate controls	Age at operation, days*	Age at inoculation, days	No. of takes on 1st inoculation	No. of regressions of 1st inoculation tumors	Result of 2nd inoculation (when 1st did not take)
1	5e	27	150	5e	5e	
2	5e	27	180	5e	5e	
3	6e	38 (4L)	150	0	0	4T, 3R
4	6e	60 (3L)	257	3e	0	1T, 1R
5	4e, 5c	70	71	7 (2e, 5c)	0	
6	5e	95	96	5e	0	
7	10e	100 (3L)	105	6e	0	1T, 1R
8	6e	105 (4L)	106	6e	0	
9	10c		110	6c	0	
10	4e	33 (1L)	87	3e	0	
11	17e, 4c	26 (5L)	72	9 (5e, 4c)	0	9eT, 9R
12	6e, 1c	24 (1L)	74	6 (5e, 1c)	0	
13	8e	31 (5L)	85	3e	0	4T, 4R
14	15e	27 (6L)	36	11e	4e	0
15	8e	27 (4L)	71	4e	1e	1T, 0R
16	6e	25 (1L)	107	5e	0	
17	11e	100 (5L)	102	11e	1e	0
18	9e	100 (5L)	104	8e	0	
19	13e	18 (4L)	98	5e	5e	
20	12c	(4L)	24	12c	0	
21	8c		Adult	8c	0	

*Averages but in no group is there more than a 10-day variation in age.
L indicates number of litters represented in the groups.
T indicates takes.
R indicates regressions.

whole or in part by the fact that an average of 20 days has been added to the thyroidectomy-inoculation period. Altogether 21 litters (or groups) consisting of 184 rats were used. There were 117 takes in the thyroidectomized rats with 39 complete regressions. There were 40 controls with 36 takes and no regressions. Special emphasis has been placed on the regression of well-established tumors because this phenomenon appears to represent a condition of maximum resistance to tumor growth. There is, however, evidence of retarded tumor growth in those thyroparathyroidectomized rats that showed no regression but merely a slowed rate of growth. Thus on the 21st post-inoculation day the average tumor diameter was 27.8 mm. in 13 non-regression thyroidectomized rats against 33 mm. in 9 littermate controls. Measurements on 7, 14, 28 and 35th post-inoculation days likewise showed larger tumors in the controls. To establish that a regression had taken place it was required that the inoculated tumor progressively reach a size of 10-15 mm. before starting to shrink. Biopsy snippings examined microscopically from 30 such tumors in the thyroidectomized rats have shown sarcoma without exception. The regressions were almost always complete by the end of the third post-inoculation week and were usually accomplished without ulceration, but in a few there was adherence to the skin and ulceration. In the biopsy specimens of regressing tumors the necrosis was often widespread and much greater than that seen in tumors of like size in control animals. The controls have been followed until the animals died or until the tumors became huge ulcerated growths.

Greatest resistance to tumor growth as manifested by complete regression is seen best when all tissues including the endocrine system have been adjusted to the thyroid-free state. Judging by inhibiting effect on tumor growth the best way to arrive at the thyroid-free state is to operate at weaning time. The time element may explain the difference in results obtained by Rohdenburg and coworkers and by Bischoff and Maxwell. There is considerable evidence that hypophysectomy retards tumor growth. Ball and Samuels[3] from observations on the growth of sarcoma produced by 1, 2, 5, 6 dibenzanthracene in hypophysectomized rats believe that the retardation of tumor growth is dependent upon the absence of the beneficial direct effect of pituitary secretion. Our experimental data as now developed suggest the possibility that the lag in tumor growth in rats from which the pituitary has been removed may be a secondary effect resulting from the absence of thyrotropic substance of pituitary origin. After both hypophysectomy and thyroidectomy growth of the animal is retarded and in both instances the question arises as to the effect of a diminished somatic growth on the inoculated tumor. McEwen and Thomson,[4] who disagree with the conclusion of Ball and Samuels found that the retardation of tumor growth was about the same in hypophysectomized rats and in rats on a semistarvation diet. They made the further observation that malnutrition is not a cause of tumor regression. Working with a Walker tumor that rarely regresses they found 1 regression in 23 control rats but none in 23 rats on a semistarvation diet. Bischoff, Maxwell and Ullmann[5] have retarded body growth by starvation and by poisoning with synthalin and heavy metals with no effect on tumor growth. Rous[6] found that the Flexner-Jobling rat carcinoma grew with equal rapidity in hosts emaciated by a restricted diet and in controls. In a series of young rats (not tabulated) in which the body weights have been held constant by heavy feeding with desiccated thyroid we found that our sarcoma in all rats increased in size until the animals died.

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[3] Ball, H. A., and Samuels, L. T., *Am. J. Cancer*, 1936, **26**, 547.

[4] McEwen, C. S., and Thomson, D. L., *Brit. J. Exp. Path.*, 1933, **14**, 384.

[5] Bischoff, F., Maxwell, L. C., and Ullmann, H. J., *Science*, 1931, **74**, 16.

[6] Rous, P., *J. Exp. Med.*, 1914, **20**, 433.

In our entire series of thyroparathyroidectomized rats inoculated with a spindle-cell sarcoma the tumors completely regressed in 33.3%. In controls none regressed.

8801 P

Gonadotropic Excretion in the Male Castrate. Effect of the Male Sex Hormones.

ROBERT T. FRANK AND U. J. SALMON.*

From the Laboratories of the Mount Sinai Hospital, New York City.

Our studies on female castrates showed a great increase in gonadotropic hormone excretion in the urine.[1] Two male castrates have now been investigated. In both patients bilateral orchidectomy had been performed for tuberculous epididymitis. In the first patient (L.D.) castration was performed 5 years ago, in the second (L.S.) 8 years ago. In the 1st patient, within 1 week of the operation, severe flushes and sweats occurred and have persisted. The 2nd has suffered from no symptoms.

Gonadotropic hormone urine determinations by the acetone precipitation method[2] were performed on L.D. for a preliminary period of 22 days. Both follicle stimulating and luteinizing factors were found in the urine. The daily output of follicle stimulating factor varied from 4 to 18 rat units per day; of lutein factor from 4 to 7½ rat units per day. In the second patient, 5 random 48-hour urine specimens were examined. The daily output of lutein factor varied from 4 to 8 R.U. The gonadotropic hormone effects produced in the test rats by the urine of these male castrates differed in no way from the effects obtained from the urine of female castrates.

In female castrates the administration of 20,000 to 25,000 R.U. of Progynon B regularly produced prompt disappearance of the gonadotropic hormone excretion from the urine with full, temporary relief of symptoms.[2] In the male castrate, L.D., 25,000 R.U. of Progynon B afforded no relief.

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* Hiram N. Vineberg Fellow in Gynecology.

[1] Frank, R. T., Salmon, U. J., and Friedman, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1666.

[2] Frank, R. T., and Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 311.

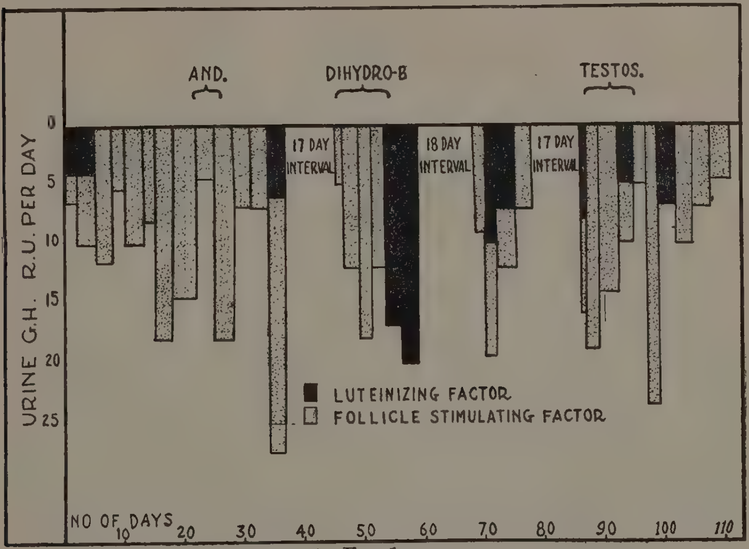

FIG. 1.

Graph of Gonadotropic Factors Excreted by a Male Castrate.
Observation period 112 days.
And. = Androsterone (20 mg.)
Dihydro-B = Dihydro-androsterone Benzoate (25 mg.) (500 capon units).
Testos. = Testosterone (115 mg.)

Several months later, L.D. was given intramuscular injections of Androsterone, Dihydro-androsterone Benzoate and later Testosterone over periods and in amounts noted on the accompanying graph. These substances were obtained through the kind cooperation of Dr. E. Schwenk and Dr. Gregory Stragnell of the Schering Corporation of New Jersey. No appreciable diminution of the gonadotropic hormone excretion was noted and only very slight improvement in the flushes was reported by the patient during the course of injection.

8802 P

Sympathetic Denervation upon Peristalsis of the Cat Small Intestine *in Situ.*

W. Burgess Sealy and S. L. Witcher. (Introduced by W. T. Dawson.)

From the Department of Anatomy, University of Texas, School of Medicine, Galveston.

We have not found in the literature any reported measurements of the effect of sympathetic denervation upon motility of the small intestine *in situ.*

In the present work 9 cats were used. The cats were kept on the same adequate diet throughout the period of observation. After the cats had been under observation for at least 10 days, they were etherized, a No. 14 F. rubber catheter passed down into the stomach, and 20 cc. of a mixture of 1 part barium sulphate to 2 parts water introduced. In about 5 minutes the cats were usually wide awake. The movements of the alimentary canal were then studied under a fluoroscope and the barium followed until the head of the column reached the caecum. This end point was usually easily determined. A total of 15 preoperative control readings was made on the 9 cats. Two of these cats died of respiratory infections prior to operation, and 1 did not recover from the later operative procedure. In the remaining 6 the lumbar sympathetic chains were successfully removed, and the splanchnic nerves sectioned below the diaphragm, which necessarily also removed the sympathetic innervation of the adrenals. The operation was completed in one stage in 3 cats, in 2 stages in 3 cats. Following complete recovery the motility of the small intestine was again studied in the manner described. The results are shown in Table I. The probability (P_t) of observed differences in results in Table II being due to chance has been calculated by Fisher's method.[1]

It will be noted that the results were obtained upon cats with some degree of sympathetic stimulation due to (1) brief etherization, (2) excitement. The stimulating effect of ether upon the sympathetic innervation of the small intestine appears to be limited, however, to the period of inhalation.[2] Since no attempt to "train" the cats to handling for the fluoroscopic observations was made it

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[1] Fisher, *Statistical Methods for Research Workers,* Section 24.1, 4th Ed., 1932, Oliver and Boyd.

[2] Bhatia and Burn, *J. Physiol.,* 1933, **78**, 257.

TABLE I.*

Cat No.	1	2	3	4	5	6	7	8	9†
Before	125(67) 105(58)	145(42) 148(33)	117(65)	132 120	130	120(40)	120 125	111(50) 123(18)	215(50) 120(18)
After	72(10) 69(14)	70(14) 71(23)	60(10) 64(14)			67(14) 70(23)		67(12) 71(14)	67(12) 68(14)

*Time in minutes for barium introduced in stomach to reach the caecum. Figures in parenthesis indicate No. of days before or after operation.

†Cat 9 on first control reading was extremely excited.

TABLE II.

	No. of cats	No. observations	Mean of observations Minutes	Standard deviation	Standard deviation of mean	P_t
Before	9	15	130	26.0	6.7	
After operation	6	12	68	3.4	.98	<.01

is considered that the grades of excitement and consequent actual or abortive sympathetic inhibition were approximately the same in the animals before and after operation. The removal of the sympathetic innervation of the adrenals tends to prevent increased epinephrine output from excitement and resultant hormonal intestinal inhibition.

Conclusion. Sympathetic denervation of the small intestine and adrenals of the cat reduces the average time for barium sulphate suspension to reach the caecum from the stomach to about 52%. The difference observed appears statistically significant.

8803 P

Influence of Magnesium Oxide on Antipyretic Action and Toxicity of Acetylsalicylic Acid in Rabbits.

David R. Climenko. (Introduced by Eric Ponder.)
From the Biological Laboratory, Cold Spring Harbor, N. Y.

Issekutz,[1, 2] and Barbour and his coworkers[3-6] have shown that

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[1] Issekutz, B., *Therap. Monatschr.*, 1915, **39**, 379.

[2] Issekutz, B., *Arch. f. ges. Physiol.*, 1913, **151**, 456.

[3] Winter, J. E., and Barbour, H. G., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 587.

[4] Barbour, H. G., and Winter, J. E., *J. Pharm. Exp. Therap.*, 1929, **35**, 425.

[5] Barbour, H. G., and Winter, J. E., Proc. Soc. Exp. Biol. and Med., 1928, **25**, 582.

[6] Winter, J. E., Richey, C. H., and Barbour, H. G., *J. Pharm. Exp. Therap.*, 1930, **38**, 3.

the simultaneous administration of magnesium compounds together with certain depressant drugs, produces a depressant action which is considerably greater than that which results from the administration of either one of these substances. Meltzer and his coworkers[7, 8] have shown that this is primarily a central, rather than peripheral action, and have demonstrated that the narcotic action of ether may be enhanced by the simultaneous administration of magnesium sulphate.

It is the purpose of this investigation to examine critically some of these statements because of their importance both from the utilitarian therapeutic point of view and from the theoretical point of view in elucidating the phenomenon of potentiation or synergism.

In dealing with such a problem, a mensurable, objective reaction which will serve as a criterion of the depressant activity is of primary importance. Also, the experimental work should cover a sufficiently large group of animals to allow for a statistical analysis of the results. This was carried out in the present investigation by measuring the antipyretic action of a given dose of acetylsalicylic acid, magnesium oxide, and of combinations of the two.

The experimental work was divided into a series of 23 experiments, using 3 groups of animals with 4 or 5 animals in each group, the total number of animals being 266. The experimental animals were all normal, healthy adult rabbits of both sexes, in which pyrexia was induced by the intravenous injection of 0.1 cc./kg. body weight of a Typhoid Toxin.* The body temperature was taken at 3 different intervals over a period of 2 hours prior to any experimental interference in order to establish a normal level for each animal. Immediately after the intravenous injection of the typhoid toxin, a stomach tube was passed and a suspension of the antipyretic drug in water was administered; in the case of the control animals the same quantity of water was passed into the stomach as was used for the suspension of the drug in the experimental animals. The body temperatures were taken at regular intervals for a period of 4 to 6 hours after this experimental interference. During this period, when observations were being made, the animals were immobilized by placing them in small, open boxes in such a manner

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[7] Meltzer, S., and Auer, J., *Am. J. Physiol.*, 1905, **15**.

[8] Meltzer, S. J., *Med. Rec.*, 1905, Dec. 16.

* Typhoid Toxin: 1 cc. represents:

B. typhosus	1000 million
B. paratyphosus A	750 ”
B. paratyphosus B	750 ”

as to minimize the effect of extraneous temperature changes which took place around them. Body temperatures were taken with a large bulb, chemical thermometer reading to 0.1°C., the bulb being left in the rectum until the column of mercury reached a maximum; this usually required about 2 minutes.

Figures 1 and 2 show the antipyretic action of the following agents compared against the pyrexia of the control animals:

a) acetylsalicylic acid (50 mg./kg.)—(each point represents the average of 46 animals).
b) magnesium oxide (50 mg./kg.)—(each point represents the average of 46 animals).
c) acetylsalicylic acid (100 mg./kg.)—(each point represents the average of 57 animals).
d) acetylsalicylic acid (50 mg./kg.) plus magnesium oxide (50 mg./kg.)—(each point represents the average of 59 animals).
e) control—(each point represents the average of 58 animals).

A statistical analysis of these results is given in Table I.

It becomes obvious from an examination of these results that the oral administration of 50 mg./kg. of acetylsalicylic acid or magnesium oxide has practically no antipyretic action, but that the antipyretic action of the combination of these 2 is as great as the antipyretic effect produced by a dose of 100 mg./kg. of acetylsalicylic acid.

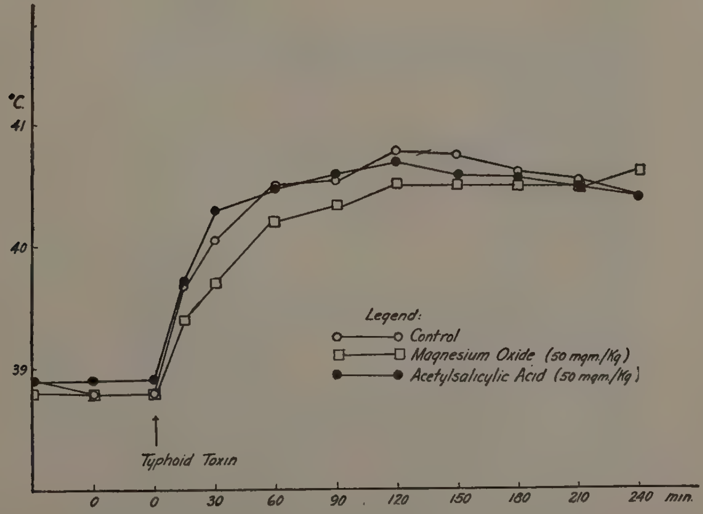

Fig. 1.

TABLE I.
Comparative Antipyretic Effect of Acetylsalicylic Acid, Magnesium Oxide, and of a Mixture of the Two.

	Group A		Group B		Group C		Group D		Group E	
Time in min.	Mean Temp.	Standard error of mean	Mean Temp.	Standard error of mean	Mean Temp.	Standard error of mean	Mean Temp.	Standard error of mean	Mean Temp.	Standard error of mean
0	38.9	.072	39.0	.059	39.0	.064	38.9	.091	38.8	.036
0	38.8	.078	39.0	.057	39.0	.060	38.9	.093	38.8	.036
0	38.8	.079	39.1	.060	38.9	.064	38.9	.112	38.8	.046
15	39.7	.064	39.2	.070	39.2	.059	39.7	.105	39.4	.054
30	40.1	.100	39.5	.067	39.4	.066	40.3	.134	39.7	.068
60	40.5	.105	39.8	.076	39.9	.055	40.5	.122	40.2	.084
90	40.5	.112	40.0	.075	40.1	.077	40.6	.134	40.3	.080
120	40.8	.108	39.7	.084	40.2	.070	40.7	.130	40.5	.076
150	40.8	.118	40.1	.088	40.2	.081	40.6	.144	40.5	.076
180	40.6	.126	40.1	.086	40.3	.084	40.6	.133	40.5	.100
210	40.6	.123	40.1	.087	40.3	.089	40.5	.088	40.5	.107
240	40.5	.110	40.2	.084	40.2	.089	40.4	.120	40.6	.086

Group A—Control series. (Each reading represents the average of 58 animals).

Group B—Acetylsalicylic Acid (50 mg./kg.) plus Magnesium Oxide (50 mg./kg.). (Each reading represents the average of 59 animals.)

Group C—Acetylsalicylic Acid (100 mg./kg.). (Each reading represents the average of 57 animals).

Group D—Acetylsalicylic Acid (50 mg./kg.). (Each reading represents the average of 46 animals).

Group E—Magnesium Oxide (50 mg./kg.). (Each reading represents the average of 46 animals).

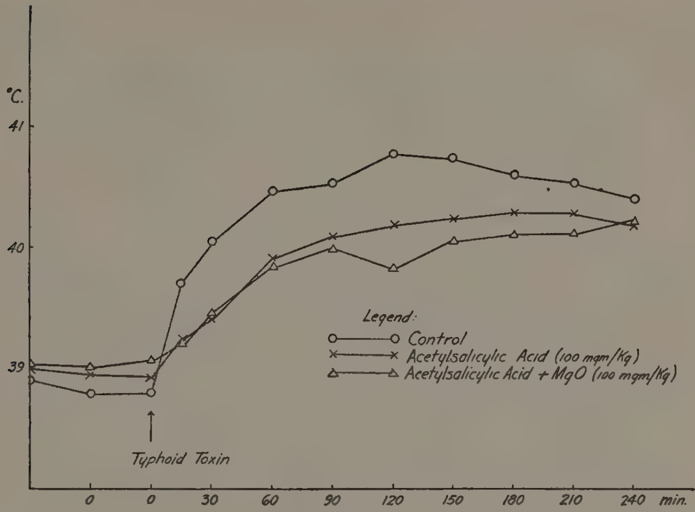

Fig. 2.

The oral toxicity of acetylsalicylic acid and of a mixture of equal parts by weight of acetylsalicylic acid and magnesium oxide is given in Table II. An examination of these results shows that the toxicity of acetylsalicylic acid is not influenced by the magnesium oxide.

TABLE II.
Toxicity (Oral) of Acetylsalicylic Acid and of Acetylsalicylic Acid Plus Magnesium Oxide.

	Acetylsalicylic Acid			Acetylsalicylic Acid (50%) + MgO (50%)		
Dose, mg./kg.	Total No. of animals	Died	Recovered	Total No. of animals	Died	Recovered
200	5	0	5	5	0	5
400	5	0	5	5	0	5
750	5	1	4	5	0	5
1000	10	5	5	10	1	9
1200	5	2	3	5	2	3
1500	10	8	2	5	2	3
1700	—	—	—	5	1	4
2000	10	10	0	5	1	4
2500	—	—	—	10	3	7
3000	—	—	—	10	8	2
Total	50			65		

Conclusions. 1. Quantities of acetylsalicylic acid and magnesium oxide, which have no antipyretic action when administered separately, have a marked antipyretic action when administered together.

2. The toxicity of acetylsalicylic acid is not influenced by the simultaneous administration of magnesium oxide. 3. The toxicity of a mixture of 50% magnesium oxide and 50% of acetylsalicylic acid is half as great as the toxicity of the amount of acetylsalicylic acid which is required to produce the same therapeutic effect.

8804 C

Incidence of Hemolytic Streptococci and Pneumococci in the Pharyngeal Flora of Normal Rhesus Monkeys.

BEATRICE CARRIER SEEGAL, GEORGE HELLER AND JACOB JABLONOWITZ.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York.

The normal bacteriologic flora of the pharynx of chimpanzees has been described by Dochez, Shibley and Mills[1] in connection with their experiments on the transmission of the common cold. The bacteria occurring in the throats of apes were similar, for the most part, to those found in human throats, although the percentile incidence varied for some organisms. Apparently, no such complete study of the pharyngeal flora of lower primates has been reported. The few reports of throat-cultures in the monkey are primarily concerned with the presence of *C. diphtheriæ* in the nasopharynx. Dold and Weigmann,[2] and Ramon and Erber[3] found diphtheria-like organisms in some monkeys, but Jungeblut[4] in 50 normal rhesus monkeys found no *C. diphtheriæ*. Dold and Weigmann reported the presence of staphylococci and streptococci but did not specify the reaction of these organisms in blood-media.

During studies of experimentally induced hemolytic streptococcal infection of rhesus monkeys, a survey of the incidence of hemolytic streptococci in the normal throats of 49 animals* was undertaken. The cultures were taken during the 2nd week of January, 1936, and within 9 days of receiving the animals in the laboratory from the dealer. A swab was rubbed firmly over the faucial surface, streaked on sheep-blood agar, and after 24 hours' incubation, the plates were searched for hemolytic colonies.

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[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., *J. Exp. Med.*, 1936, **52**, 701.

[2] Dold, H., and Weigmann, F., *Zeitsch. f. Hyg.*, 1934, **116**, 154.

[3] Ramon, G., and Erber, B., *Revue D'Immunologie*, 1935, Sept., 415.

[4] Jungeblut, C. W., *J. Imm.*, 1934, **27**, 17.

* These monkeys were made available through the courtesy of Dr. Jungeblut.

From 28 of the 49 cultures a β-streptococcus[5] was isolated. On one plate a minute streptococcus of the type described by Bliss and Long[6] was also isolated. These organisms were tested for production of a soluble hemolysin, lysis of human and rhesus plasma-clot (Tillett and Garner[7]) and fermentation of lactose, mannite, and salicin. The antigenic group to which the organisms belonged was determined by the method of Lancefield.[8] Six of the strains were tested for the production of a toxin reactive in the skin of silver-fox rabbits.

The results of these tests are summarized in Table I: 19 of the strains fell in Group A, 4 in Group C, 5 in Group G, and 1, the minute streptococcus in Group F.

All Group A organisms were *Streptococcus pyogenes* by Holman's classification.[9] The diameter of hemolysis in rabbit-blood agar† pour-plates was 2.5 mm. A soluble hemolysin active against rabbit red blood cells was produced and a fibrinolysin which dissolved human-plasma clot within 3½ hours. The lysis of monkey-plasma clot required a longer time, for it was complete within 6 hours of initial observation in the case of only 3 strains. Seven of the strains produced complete lysis within 22 hours, another 7 were partially lytic in 24 hours, and 2 produced no lysis in 24 hours. A toxin active in the skin of silver-fox rabbits was formed by all the 6 strains tested.

The 4 strains of hemolytic streptococci belonging to Group C resembled the organisms of Group A in sugar-fermentations and elaboration of a soluble hemolysin and fibrinolysin. The diameter of hemolysis in pour-plates was 4 mm.

One of the 5 strains of hemolytic streptococci belonging to Group G was *pyogenes* and the other 4 were fermentatively *equi*. The diameter of colonial hemolysis in a rabbit-blood pour-plate was 4 mm. Their fibrinolytic and hemolytic activity was similar to that of the members of Groups A and C.

The streptococcus of Group F, *equi,* produced the characteristically small area of hemolysis in pour-plates with an almost micro-

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[5] Brown, J. H., *Mono. Rockefeller Inst. Med. Res.*, 1919, **9.**

[6] Bliss, E. A., and Long, P. H., *J. Bact.*, 1934, **37**, 105.

[7] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[8] Lancefield, R., *J. Exp. Med.*, 1933, **57**, 571.

[9] Holman, W. L., *J. Med. Res.*, 1916, **34**, 377.

† This medium contained 1% neopeptone.

TABLE I.

Cultural and Immunological Characteristics of Hemolytic Streptococci Isolated from the Throats of Normal Rhesus Monkeys.

No. of Strains	Fermentation			Pour plate; diam. of hemolyzed zone	Soluble hemolysin	Group	Fibrinolysis; human clot; time for lysis	Fibrinolysis; rhesus clot; time for lysis	Toxin
	Lactose	Mannite	Salicin						
19	+	0	+	2.5 mm.	+	A	19 min. to 3½ hrs.	3 hrs. to 0 in 24 hrs.	All 6 tested positive
4	+	0	+	4 mm.	+	C	19 min. to 3¼ hrs.	2¼ to 24 hrs.	
1	+	0	+	4 mm.	+	G	1½ hrs.	25% in 6 hrs. 100% in 24 hrs.	
4	0	0	+	4 mm.	+	G	1¼ to 2 hrs.	10% to 100%, 24 hrs.	
1	0	0	+	1 mm.	0	F	None, 24 hrs.	None, 24 hrs.	

scopic rosette colony. No soluble hemolysin or fibrinolysin was formed.

Of the colonies on the original plates a large number resembled pneumococcal colonies. Typable pneumococci were isolated from 33 of the 49 cultures: 3 type III, 9 type IV, 1 type V, 15 type VII, 1 type VIII, 3 type XIX, and 1 type XXXII. A rough pneumococcus agglutinating in all antipneumococcal sera was obtained from 2 cultures and a third culture yielded a pneumococcus which failed to agglutinate in any of the 32 type-sera.

A single throat-culture from a group of 49 normal rhesus monkeys yielded hemolytic streptococci in 29 and type-specific pneumococci in 33 cases. Nineteen of the 29 hemolytic streptococci belonged to human Group A, 4 to Group C, 5 to Group G, and 1 to Group F. The pneumococci belonged to types III, IV, V, VII, VIII, XIX, and XXXII, with types IV and VII predominating. These organisms were present in considerable numbers. Some of the plates contained as many as 50 isolated hemolytic colonies and the pneumococci also were well represented.

It is noteworthy that hemolytic streptococci isolated from the throats of monkeys are better able to lyze human than monkey-plasma clot. In this respect they are similar to the human microorganisms studied by Van Deventer and Reich.[10] It is also to be noted that the representatives of Groups C and G were fibrinolytically similar to Group A. These findings diverge from those of Madison,[11] who found that hemolytic streptococci isolated from diseases of animals were usually specifically lytic for their clotted plasma and suggested that the fibrinolysis of human plasma was associated specifically with Group A streptococci. Tillett[12] also reported that of 21 Group C organisms tested, only one was fibrinolytic for human plasma. Hare,[13] on the other hand, found all of 15 Group C and 9 of 13 Group G cocci fibrinolytic for human plasma; this agrees with the results obtained with our strains from monkeys. Possibly the interval between isolation and testing for fibrinolysis accounts for these differences, and the host from which the Group C and G organisms were obtained may influence the results also.

The animals studied had been in captivity for some time and it is therefore possible that they had acquired the hemolytic streptococci and pneumococci from their human contacts. Irrespective of the source of these organisms, it is apparent from their numbers that they found a favorable environment in the pharynx of the monkeys. Occasional hemolytic streptococcal infections have occurred in handlers of monkeys and the findings suggest that the rhesus monkey may transmit the Group A organism to man.

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[10] Van Deventer, J. K., and Reich, T., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 821.

[11] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 444, 49.

[12] Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

[13] Hare, R., *J. Path. and Bact.*, 1935, **41**, 499.

We wish to express our thanks to Miss M. Olmstead of the Department of Medicine who helped us with the Lancefield grouping.

8805 P

Action of the Lytic Principle of Pneumococcus on Certain Tissue Polysaccharides.

KARL MEYER, RENÉ DUBOS AND ELIZABETH M. SMYTH.

From the Department of Ophthalmology, College of Physicians and Surgeons; the Institute of Ophthalmology, Presbyterian Hospital, and the Hospital of the Rockefeller Institute, New York.

Avery and Cullen[1] obtained from both rough and smooth pneumococci a ferment capable of lysing heat-killed pneumococci. In unpublished experiments (Dubos) it was found that the same ferment preparation renders ineffective in rabbits the capsular type specific antigen of killed cells of virulent pneumococci.

A hydrolytic action of the ferment preparation of pneumococcus on two tissue polysaccharides is described here. This action seems to be identical with that of the same ferment on the pneumococci.

From bovine vitreous humor and from human umbilical cord two polysaccharides have been obtained in pure form, seemingly identical in composition, rotation, and general physical behavior.[2] They are composed of acetylglucosamine and glucuronic acid, the components having been shown to be present in equimolar ratio by isolation or quantitative analysis.

The 2 polysaccharide acids yielded reducing sugar following hydrolysis by the ferment obtained from pneumococcus autolysates. The hydrolysis is optimal between pH 5 and 6, and does not take place above pH 8 nor below pH 4.5. The action of the ferment on killed pneumococci has about the same pH optimum. The action on both the pneumococci and the 2 polysaccharide acids is abolished

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[1] Avery, O. T., and Cullen, G. E., *J. Exp. Med.*, 1923, **38**, 199.

[2] Meyer, Karl, and Palmer, John W., *J. Biol. Chem.* (in press).

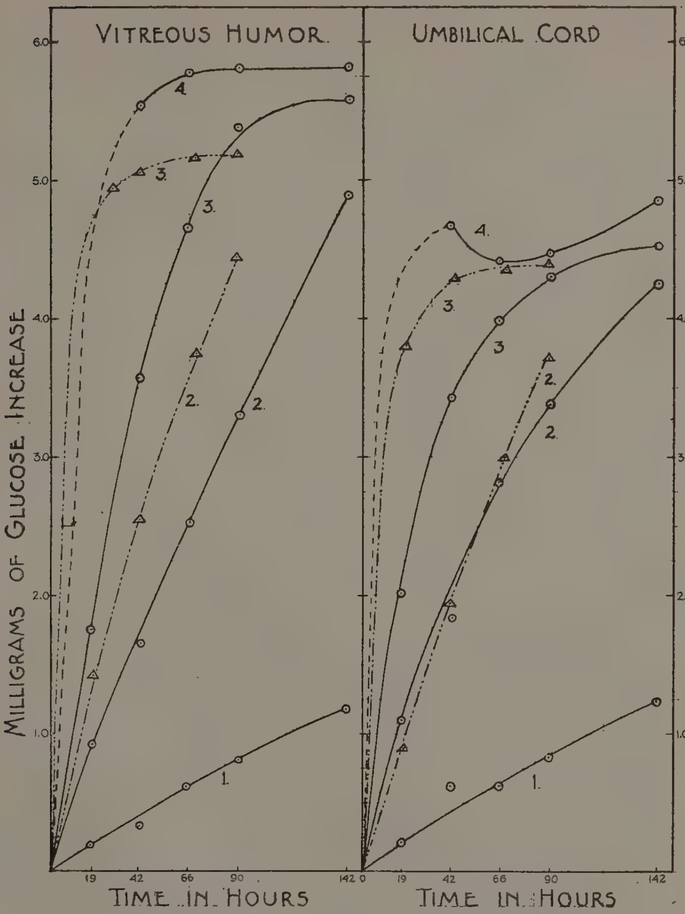

FIG. 1.

Solid lines represent experiment I; broken lines, experiment II. Numbers denote concentration of ferment solution: 1, 0.05%; 2, 0.10%; 3, 0.50%; 4, 1.00%.

by heating the ferment or by treating it with iodine. The iodine inactivation is reversed by sulfite and to a lesser degree by arsenite, as measured by the polysaccharide hydrolysis.

Further evidence that the same principle is active against the 2 tissue polysaccharides and the pneumococci is found in the following inhibition reaction: 2.0 cc. of a 0.1 percent pneumococcus ferment solution in phosphate buffer at pH 6.0 were incubated for 6 hours with 10 mg. of polysaccharide (vitreous and umbilical cord). When tested against a suspension of heat-killed pneumococci at the end of the incubation period, the ferment was found to have lost at least 90% of its lytic activity. The control ferment solution also incubated at pH 6.0 for the same length of time in the absence of the polysaccharide retained all its activity.

The enzymatic hydrolysis of the polysaccharide yields about 70% of the theoretical amount of reducing sugar. The reaction appears specific, since commercial trypsin, saliva, taka-diastase and emulsin do not hydrolyze the carbohydrates. The pneumococcus ferment, on the other hand, is without action on chondroitin sulfuric acid or on a polysaccharide isolated from gastric mucin[3] containing acetylglucosamine and galactose.

So far the only other source of an enzyme capable of hydrolyzing the two polysaccharides is a tissue hash from rabbit iris and ciliary body. Since aqueous humor also contains the polysaccharide acid,[4] it is probable that the ferment plays a rôle in the fluid exchange of the eye.

Typical results of the hydrolysis of the polysaccharides by the pneumococcus enzyme are shown in the text figure. Ten mg. amounts of the acids in 1.0 cc. of citrate buffer, pH 6.0, were incubated with 1.0 cc. of respectively 0.05, 0.1, 0.5, and 1.0 percent solutions of pneumococcus ferment. Two drops of toluene were added. As controls the same concentration of the acids were incubated with 1.0 cc. of saline and 1.0 cc. of citrate buffer with 1.0 cc. of ferment. Reducing sugar was estimated on aliquot portions of the mixture by the Hagedorn-Jensen method. The control values were subtracted.

8806 C

Attempt to Produce Atherosclerosis in Chickens by Feeding Cholesterol.*

H. D. KESTEN, D. R. MEEKER AND J. W. JOBLING.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

In view of the inability of various workers throughout the world

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[3] Meyer, Karl, Palmer, John W., and Smyth, Elizabeth M. (unpublished experiments).

[4] Meyer, Karl, and Palmer, John W., *Am. J. Ophthal.* (in press).

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation.

to produce experimental arteriosclerosis comparable in pathogenesis and pathology to that occurring in man, other animals besides the commonly used rabbit have been under investigation in this laboratory. One of these is the chicken, accustomed to ingesting and excreting cholesterol, in contrast to the rabbit, which is herbivorous. This report covers an attempt to produce atherosclerosis in chickens by the administration of cholesterol in large amounts.

Duff,[1] in a recent review of experimental cholesterol arteriosclerosis, found insufficient evidence that cholesterol feeding affects the arteries of chickens. Yamaguchi[2] has reported the occurrence of atherosclerosis in the aorta of chickens fed egg yolk or lanolin over long periods, and Uchiyama[3] believed that the aortic intimal changes observed following cholesterol feeding were more advanced than in control chickens, but emphasis remains on the frequent spontaneous occurrence of lipid-containing fibrous lesions in the intima and inner media of the aorta of relatively old chickens. Younger birds, in which spontaneous vascular lesions presumably should be relatively infrequent, have not been used for this type of experiment.

Further support of Uchiyama's conclusions, however, was obtained when cholesterol was fed to 17 five-months-old White Leghorn roosters. Nine of the 14 were fed daily for 1½ to 4 months 0.3 gm. of cholesterol attached to scratch corn by dissolving in ether and a little olive oil, and stirring with the grain until the ether had completely evaporated. (The basic diet of this group consisted of mixed scratch grain and oats and was cholesterol free). The remainder received 0.8 gm. or 1.6 gm. doses of cholesterol mixed with olive oil and administered by capsule 4 times a week tor 2½ to 3 months. (The diet of these chickens was composed of scratch grain and egg mash, the latter adding approximately 0.03 gm. of cholesterol to the daily intake. This diet was used for the second group of chickens because of the difficulty experienced in keeping the first group in good condition in the laboratory on a grain diet alone). An elevation of serum cholesterol (up to 300 mg. per 100 cc.; normal: 80-160) was produced in only ⅓ of the fowls, indicating inadequate absorption, or, more probably, the ability of the omnivore to metabolize cholesterol. Seven of the 14 roosters, when killed at the end of the feeding period, had small yellow longitudinal plaques or streaks in the abdominal or muscular portion of the aorta, consisting of intimal fibrosis and thickening, with infiltration of the thickened intima by fine and coarse droplets of lipid, which was in part doubly refractive. Some of this fat was within large mononuclear phagocytes. There were also more or less fan-shaped radiating deposits of similar lipid extending outward in the media beneath the intimal lesions with occasional interruption of the internal elastic lamella (Fig. 1). When these deposits were extensive a few muscle cells had been replaced by the accompanying fibrous tissue. In 3 other chickens grossly invisible patches of fibrosis of the intima without stainable fat were noted in sections. There was no correlation between the arteriosclerotic change and the blood cholesterol concentration, but somewhat less lipid was noted in the aortas of the birds treated only 1½ months. Gross lesions were absent from the hearts of all birds.

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[1] Duff, G. L., *Arch. Path.*, 1935, **20**, 81 and 259.

[2] Yamaguchi, M., *Hokuetsu Igakukwai Zasshi*, 1922, **37**, quoted by Kawamura, R., *Neue Beiträge zur Morphologie und Physiologie der Cholesterinsteatose*, Jena, Gustav Fischer, 1927.

[3] Uchiyama, T., *Virch. Arch. f. Path. Anat.*, 1930, **277**, 642.

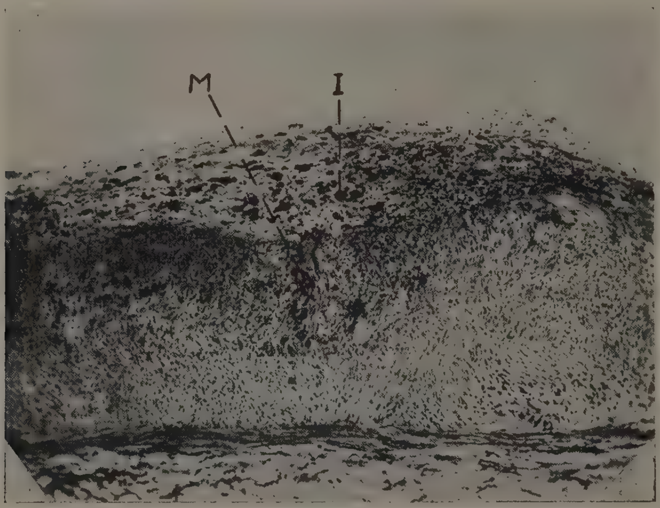

FIG. 1.
Aorta of rooster fed cholesterol, showing intimal sclerosis; also lipid in intima (I) and underlying media (M).

Twenty-five control roosters of the same age were kept on the same standard laboratory diets (9 received the cholesterol free diet, 16 that containing egg mash) but were fed no additional cholesterol. Areas of intimal fibrosis were present in the abdominal aorta of 14 and these were accompanied by lipid deposits in 9 (Fig. 2). Fat

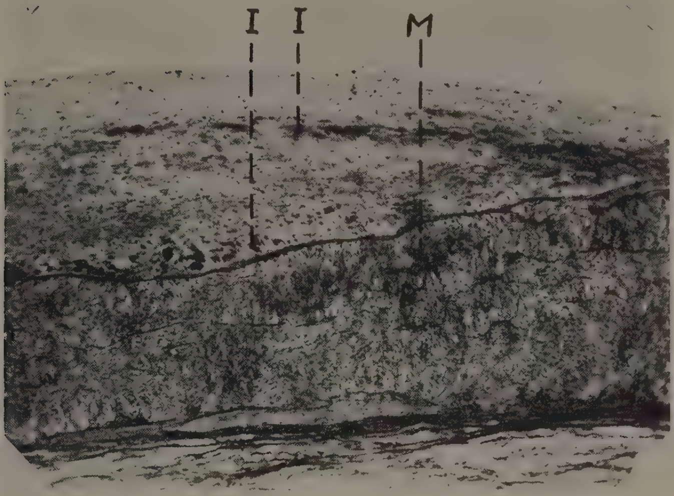

Fig. 2.
Same in control chicken. (Combined Weigert-Sudan stain.)

was present in the underlying media in only 4, however, and these medial deposits were rather less extensive and without the fan-shaped radiating distribution suggestive of rapid infiltration from the intima noted in some of the cholesterol-fed birds. Small deposits of lipid were present in the mid-portion of the media, unassociated with an intimal plaque, in the thoracic aorta of one control chicken, but no other independent medial lesions were observed.

Conclusion. The aorta of the young adult rooster commonly exhibits areas of intimal fibrosis, with an infiltration of lipid into the thickened intima and the underlying media. Feeding of cholesterol for periods up to 4 months fails to increase the incidence of such lesions, but may slightly augment the deposition of lipid, particularly in the media. For the production of intimal lipid deposits in the aorta by feeding cholesterol, the chicken, therefore, appears to be an unsatisfactory animal.

8807 C

Susceptibility of the "Gopher," *Citellus Richardsonii* (Sabine), to Equine Encephalomyelitis.

Jerome T. Syverton and George Packer Berry.

From the Department of Bacteriology, University of Rochester, School of Medicine and Dentistry, Rochester, New York.

Epizootic equine encephalomyelitis, the specific causative virus of which was first recovered from affected horses by Meyer, Haring and Howitt,[1] is a disease with a definite seasonal occurrence and an advancing geographical distribution in North America.[2] Two distinct types of the virus have been recognized, Western and Eastern[3, 4], of which the latter is restricted to the Atlantic coast states, whereas the former is spreading over an increasingly large area.[2] The disease is limited in nature, insofar as present knowledge is concerned, to members of the equine family, although evidence somewhat suggestive of its transmission to man has been presented by Meyer.[5] Experimentally, the susceptibility of a wide variety of hosts has been demonstrated.[1,2,4,6,7,8,9] (The host range is more limited for the Western than for the Eastern type.) In spite of this fact, however, no possible residual host has as yet been demonstrated among the wild animals native to North America. The discovery of such a host would be noteworthy, especially if the new host were representative of a large number of wild animals with natural habitats in close proximity to equines during the limited time of year at which the disease occurs. Evidence that such a host has been found is offered in the present communication, which reports the susceptibility of the "gopher," *Citellus richardsonii* (Sabine),* to the virus of equine encephalomyelitis, Western type.† This type was used because the geographical distribution of the disease in equines embraces that of the newly demonstrated host. Furthermore, the lower virulence of this type of virus lends greater significance to the successful serial passage by intranasal instillation of virus.

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[1] Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227; *J. Am. Vet. Med. Assn.*, 1931, **79** (N.S. **32**), 376.

[2] Giltner, L. T., and Shahan, M. S., *J. Am. Vet Med. Assn.*, 1936, **88** (N.S. **41**), 363.

[3] Ten Broeck, C., and Merrill, M. H., Proc. Soc. Exp. Biol. and Med., 1933, **31**, 217.

[4] Giltner, L. T., and Shahan, M. S., *Science*, 1933, **78**, 63.

[5] Meyer, K. F., *Ann. Int. Med.*, 1932, **6**, 645.

[6] Syverton, J. T., Cox, H. R., and Olitsky, P. K., *Science*, 1933, **78**, 216.

[7] Olitsky, P. K., Cox, H. R., and Syverton, J. T., *J. Exp. Med.*, 1934, **59**, 159.

[8] Hurst, E. W., *J. Exp. Med.*, 1934, **59**, 529.

[9] Remlinger, P., and Bailly, J., *Bull. Acad. Vet. France*, 1935, **8**, 420.

* We wish to thank Mr. George G. Goodwin of the American Museum of Natural History, New York City, for specific allocation of the "gophers" used.

The Richardson ground squirrel, *Citellus richardsonii* (Sabine), is native to Saskatchewan, Alberta, Montana and the Dakotas. It is known locally by the name "gopher." Probably the most common wild animal in this district, it represents one of a large number of closely related species which are distributed throughout much of the United States and Canada. Its natural habitat is pastures and fields where grain, roots and other vegetation provide ample food supply. The adult animal, gray-brown in color, measures from 7 to 9 inches over all and is characterized, as are the related species, by the possession of long claws and powerful limbs with which to burrow beneath the surface of the ground. Several litters of from 2 to 8 offspring each are born during the summer months. This explains the great increase in numbers as well as the marked activity of the animals during this period.

The brain tissue of a guinea pig *in extremis* following the intranasal instillation of the Western type of equine encephalomyelitis virus was used as source virus. This material was found to be active in the guinea pig brain in a dilution of 10^6. The tissue was ground without an abrasive and diluted to make a 10% suspension in nutrient broth. Following centrifugation at 1500 r.p.m. for 5 minutes, the supernatant fluid was introduced into the nares of a "gopher" under light ether anesthesia. Introduction of the fluid was accomplished by means of a tuberculin syringe fitted with a blunt needle, care being taken to prevent the needle from touching the tissues. Using a similar procedure, the inoculum consisting of brain tissue of the generation preceding, the infectious agent was carried through 12 successive passages in "gophers." The clinical syndrome, which terminates fatally in most instances, is characterized by an incubation period of from 2 to 4 days, followed by general weakness, ataxia, marked tremors, grinding of the teeth, with death on the third to sixth days. Thus it is similar to that observed in domesticated laboratory rodents. It differs from that of the guinea pig and rabbit in that less than 50% show a febrile response.

Following 12 successive serial passages in "gophers" by intranasal inoculation of virus-brain tissue suspension derived from the preceding passage host, the virus was found to be lethal in a dilution of 10^4. The titer was determined by the intracerebral inoculation of guinea pigs, using two animals for each decimal dilution. This titer does not differ materially from that in guinea pigs following serial passage by the intranasal technique.

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† Samples of the Eastern and Western types of equine encephalomyelitis virus were sent to us through the kindness of Doctors L. T. Giltner and W. S. Gochenour of the United States Bureau of Animal Industry, Washington.

Following the "gopher" passage, the specificity of the passaged virus was verified by its specific neutralization with rabbit anti-equine encephalomyelitis serum of the Western type.‡ Briefly, the technique consisted of mixing equal parts of a 2% broth suspension of "gopher" brain virus with an equal volume of anti-equine encephalomyelitis rabbit serum and normal rabbit serum for control. The serum-virus mixtures were incubated at 37°C. for 2 hours and used in 0.15 cc. amounts for the intracerebral inoculation of guinea pigs under light ether anesthesia. Two animals were used for each decimal dilution of a series extending from 10^1 through 10^7. The antiserum protected all of the animals with the exception of one receiving the 10^2 mixture. All of the control animals died up to and including the pair which received 10^4.

It is noteworthy that one "gopher" was found to be refractory to the disease. This suggested that this animal may have contracted the disease in nature. With this possibility in mind, cage contact was attempted. To date none of the small number of contact animals tested has contracted the disease. Work is under way to determine the susceptibility of other native wild rodents as well as the possible rôle of arachnid vectors in the dissemination of this disease.

Summary.—Evidence is presented of the susceptibility of the "gopher" or Richardson ground squirrel, *Citellus richardsonii* (Sabine), to the virus of equine encephalomyelitis, Western type. Twelve successive passages through "gophers" have been effected by intranasal instillation of virus-brain tissue suspensions derived from the preceding passage. The virus was recovered following the final passage. Its pathogenicity for guinea pigs and its specificity were demonstrated. It is suggested that the "gopher" and other wild rodents may possibly act as reservoir hosts for the virus in nature.

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‡ We are indebted to Doctor H. R. Cox of the Rockefeller Institute for Medical Research, New York City, for supplying us with rabbit anti-serum against both the Eastern and Western types of equine encephalomyelitis virus. This enabled us to establish the serological specificity of the gopher-passaged virus with antisera prepared against different lots of virus than that used for initiating the present studies.

8808 P

Inhibition of Gastric Peristalsis Due to a Substance Found in Commercial Peptones.

J. Earl Thomas and J. O. Crider.

From the Department of Physiology of the Jefferson Medical College of Philadelphia.

In previous communications one of us[1,2] called attention to the importance of gastric inhibition due to stimuli acting in the duodenum, in the regulation of gastric emptying. In the case of acid and certain other chemical stimuli the inhibition was found to be due to an "enterogastric" reflex over the vagus nerves. Later the present authors with Dr. Mogan[3] reported additional experimental evidence leading to the same conclusions and added proof that the normal duodenal contents following a meal of raw lean beef act as adequate stimuli to cause gastric inhibition. Experiments were reported which indicate that the inhibitory effect is in part due to chemical stimuli, the nature of which was not determined. The present study is an effort to identify the inhibitory agent.

The type of animal preparation and the methods used are described in the previous reports.

Brunemeier and Carlson[4] observed that 10% Witte's peptone in 0.2% HCl, when injected into the duodenum, stopped gastric hunger contractions. In experiments performed in July, 1935, we attempted to use Witte's peptone as a buffering agent for HCl solutions to be used in a further study of gastric inhibition from the duodenum. We found that the peptone, even in neutral solution, caused inhibition of gastric peristalsis.

In those experiments, after a meal of raw lean beef, the duodenum (of dogs) was perfused through its lumen with various solutions, using normal saline as a control. Fig. 1 (Exp. of July 24, 1935,) shows the effect on antral peristalsis of changing the perfusion fluid from normal saline to 0.5% Witte's peptone in neutral solution.

A further study of this phenomenon has been made by injecting 10 cc. quantities of distilled water solutions of various commercial

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[1] Thomas, J. Earl, *J. A. M. A.*, 1931, **97**, 1663.

[2] Thomas, J. Earl, and Mogan, C. J., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 968.

[3] Thomas, J. Earl, Crider, J. O., and Mogan, C. J., *Am. J. Physiol.*, 1934, **108**, 683.

[4] Brunemeier, E. H., and Carlson, A. J., *Am. J. Physiol.*, 1915, **36**, 191.

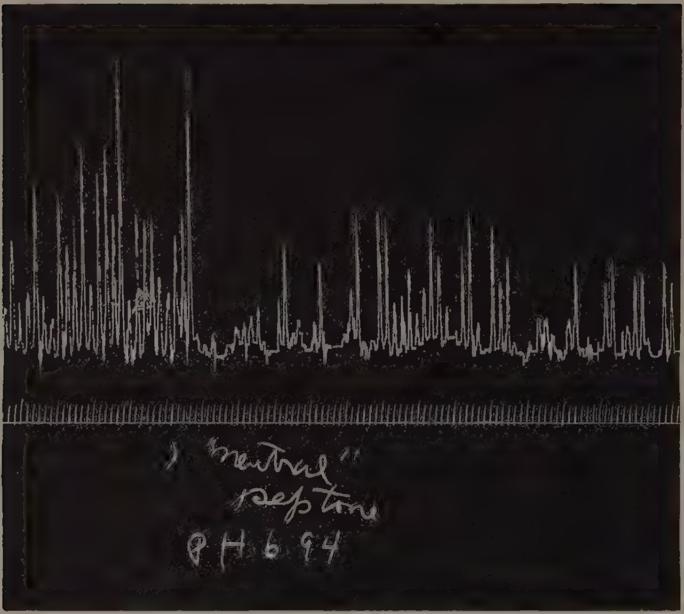

FIG. 1.

Balloon record of antral peristalsis during perfusion of the duodenum. Perfusion fluid was changed at the point indicated from normal saline to 0.5% Witte's peptone in neutral solution in normal saline. Time in 10-second intervals. Bromoform manometer.

peptones into the first part of the duodenum of dogs with the stomach empty or shortly after feeding a meal of dog biscuit. Witte's peptone, "Proteose" peptone, "Bacto" peptone and a granular peptone produced by a French manufacturer have been investigated.

Witte's peptone caused gastric inhibition from the duodenum in 2% solution, "Proteose" peptone in 5%, and "Bacto" peptone in 10% solution. "Proteose" peptone in 2% and "Bacto" peptone in 5% solution gave questionable reactions. In 2% solution "Bacto" peptone and the French peptone were without effect. The French peptone was not investigated further.

Solutions of Witte's peptone and of "Bacto" peptone were saturated with ammonium sulphate and the redissolved precipitate investigated. The precipitate is assumed to consist mainly of proteoses. The proteose solutions were approximately as effective as the corresponding solutions of the whole peptone mixture from which they were obtained, making some allowance for loss in the manipulations necessary to free the preparations from adherent ammonium sulphate. We conclude that the active substance is mainly proteose or is precipitated with it.

Further evidence that the inhibitory substance is one of the higher derivatives of protein digestion appears when our results are compared with chemical data kindly loaned us by Dr. Carl J. Bucher. This comprises an analysis of various commercial peptones for protein nitrogen, non-protein nitrogen, polypeptid nitrogen, amino acids, salt and sugar. The inhibitory effect of the various preparations varies directly with the amount of "protein" nitrogen, inversely with the amount of non-protein nitrogen and polypeptid nitrogen, and is in no way related to the total nitrogen. The analysis shows an insignificant amount of salt and amino acid in the most effective preparations and no sugar (a possible source of error) in Witte's peptone.

We conclude that certain products of protein hydrolysis, probably proteoses, act in the intestine to inhibit gastric peristalsis. The observation has a bearing on the regulation of gastric emptying during protein digestion and completes the list of major foodstuffs, each of which has now been shown to be capable of inhibiting gastric activity when present in the intestine in adequate concentration and in certain stages of digestion.

Further work is contemplated directed toward the isolation of similarly active material from the products of normal digestion in the animal and to learn whether the inhibitory effect is reflex, humoral, or both. Present indications point to a reflex effect inasmuch as the latent period for inhibition is often less than 30 seconds.

8809 P

Creatine Content of Human Hearts.

GEORGE HERRMANN, GEORGE M. DECHERD, JR., AND TOM OLIVER.
From the Departments of Medicine and Pathology, University of Texas.

Even before the important rôle of phosphocreatine in muscular contraction was recognized, Constabel[1] noted that there was a decreased total creatine content in those human hearts that showed either clinical or post-mortem evidence of damage. Recently

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[1] Constabel, Fr., *Biochem. Z.*, 1921, **122**, 152.

Cowan[2] has examined a larger group of hearts, and found that myocardial failure is accompanied by a creatine content that is markedly below normal. Seecof, Linegar and Myers[3], whose method for determining myocardial creatine we have employed, have also examined a series of human hearts, emphasizing particularly the higher creatine concentration in the left ventricular muscle as compared to the right ventricle.

In our laboratory we have approached from several experimental angles the question of the chemical changes that initiate or accompany heart failure. Since our primary interest is clinical, we have also analyzed muscle from the left ventricle of human hearts, as this material became available from time to time.[4] In Table I we have summarized creatine determinations on 105 additional human hearts. The total creatine has been determined, the total solids, and from this calculated the percentage of creatine on a dry basis. The normal values have been determined in hearts from patients that died a traumatic or surgical death, and in hearts from those who died from an infectious disease, yet showed no gross or microscopic evidence of myocardial involvement. We list the mean value, with the standard deviation from the mean.

TABLE I.
Creatine Content of Human Hearts.

No.	Cause of death	Mg. %	Solids %	Dried mg. %
11	Traumatic, normal hearts	183 ± 17	20.7 ± 0.62	887 ± 79
23	Infectious normal hearts	172 ± 15.4	20.1 ± 0.98	858 ± 70
34	Normal hearts	175 ± 20.7	20.3 ± 0.87	868 ± 70
	Heart disease without failure			
1	Rheumatic	210	19.9	1058
6	Hypertension	198 ± 15.6	20.4	970 ± 79
11	Coronary sclerosis	157	20.	783
18	Total without failure	173	20.1	860
6	Glomerulonephritis with uremia	159	20.3	781
5	Severe anemia	157	19.3	819
32	Congestive heart failure	122 ± 20.8	20.3 ± 1.2	605 ± 100
10	Infections with microscopic myocardial change	121	20.5	596

In hypertrophied hearts, which have not failed, the values for total creatine are elevated; in those with myocardial change secondary to coronary sclerosis, the averages are low. The creatine content of hearts that have failed is reduced, averaging 30% below normal. Low values were also obtained in hearts that showed microscopic changes, inflammatory or degenerative, from patients that had died of infectious disease.

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[2] Cowan, D. W., *Am. Heart J.*, 1934, **9**, 378.

[3] Seecof, D. P., Linegar, C. R., and Myers, V. C., *Arch. Int. Med.*, 1934, **53**, 574.

[4] Herrmann, George, Decherd, George M., and Schwab, E. H., *Southern Med. J.*, 1936, **29**, 386.

We have previously noted[5] the drop in creatine content of infarcted heart muscle with experimental animals. Four human cases have shown the same changes. Table II shows the creatine values for the infarcted areas from human hearts, compared with the values from non-infarcted portions of the heart.

TABLE II.
Coronary Occlusion.

	Creatine mg. %		Solids %		Dried mg. %	
Ht. Wt.	Good	Infarcted	Good	Infarcted	Good	Infarcted
610	122	41	23.45	18.0	520	228
350	104	61	21.95	15.05	497	405
800	100	52	19.7	18.2	558	318
900	151	31	19.15	17.25	788	180

8810 C

Embolic Staphylococcus Arthritis and Endocarditis in Rabbits.

GENE H. KISTLER. (Introduced by A. D. Keller.)

From the Department of Pathology and Bacteriology, University of Alabama Medical School.

There have been many clinical and experimental studies of arthritis and its associated conditions. The results and hypotheses set forth, however, are confusing and conflicting, especially regarding etiology and mechanism of production. Experimental inflammatory reactions have been produced within joints by intra-articular and intravenous injections of virulent and attenuated microorganisms, bacterial toxins and various chemical agents. It has been postulated by various authors that the localization of a focus may be influenced by trauma, allergic responses and by the particular structure of joint tissues.

Studies of the blood supply and infarction of the femur in rabbits[1] demonstrated that a suspension of charcoal and agglutinated staphylococci, living or dead, can act as arterial emboli and produce septic or aseptic infarcts in bone. These infarcts occurred regularly beneath epiphyseal and articular cartilages due to end-capillaries between ossifying columns of calcified matrix and to relative poor collateral circulation to subchondral tissues. Since some of the septic infarcts were accompanied by exudative arthritis, it seemed desirable to investigate further the relationship of joint infection to embolic disturbances in contiguous bone and cartilage structures.

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[5] Decherd, G., Herrmann, G., and Davis, O., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 547; Herrmann, G., and Decherd, G., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1304.

Healthy growing rabbits (500 to 2000 gm.) were given intravenous (ear vein) injections of fully virulent, partially sterilized and killed agglutinated and non-agglutinated saline suspensions of a hemolytic *staphylococcus aureus* made from 24-hr. agar-slant cultures. The organism was obtained from the blood stream of a man with a subperiosteal abscess. Agglutination and partial or complete sterilization were produced by one to 5 drops of N/10 sulphuric acid per cubic centimeter of suspension. The amounts injected, singly or in divided doses, ranged from 0.0025 (virulent) to 1.5 cc. (killed suspension) depending upon the size of the animal, the effects desired and upon the amount of acid and the time added before injection. The animals died or were killed from 1 to 115 days after the first injection and all abnormal bone, joint and viscera tissues were taken for histologic study. A pure growth of staphylococcus, similar to the strain injected, was obtained from all foci cultured.

All the rabbits that received living organisms and survived the septicemia more than 2 days developed marked exudative arthritis in one or more joints with destruction of articular cartilage and subchondral bone. The joints were distended markedly by exudate and many had associated necrosis and separation of one or both adjacent epiphyses. The joints most commonly affected were the knee, hip and ankle. In the more chronic experiments the joints tended to heal; in a few this was complete but with residual rough articular surfaces, thick fibrous capsules and limited motion. There were also gross lesions of the heart, especially the mitral leaflets, in 53% of the animals. These ranged from small endocardial vegetations to marked suppurative pericarditis. The parenchymatous visceræ had no significant changes or a few scattered abscesses not larger than 2 mm. in diameter. This was conspicuous by contrast with the changes in the joints and structures of the heart. One animal that received 0.1 cc. of a partially sterilized and agglutinated suspension in a single dose and survived 11 days, had 21 joint and 2 endocardial foci without changes in the liver and with only 3 miliary abscesses of the kidneys. Rabbits that died within 2 days after injection had swollen, hyperemic parenchymatous visceræ, often hyperemia of the epiphyses, increased sanguinous joint fluid and hemolysis staining of the tissues generally.

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[1] Kistler, Gene H., *Arch. Surg.*, 1934, **29**, 589; *Surg., Gynec. and Obst.*, 1935, **60**, 913.

The most marked and numerous focal changes were obtained with suspensions of cocci well agglutinated and partially or almost completely sterilized by the acid, which permitted relatively large single and repeated doses and the production of chronic lesions. It was difficult to judge the dose of virulent non-agglutinated organisms for amounts as small as 0.0025 cc. produced death without localized suppuration. Sterile agglutinated and non-agglutinated suspensions aggregating 1.5 cc. produced no significant changes in any tissues.

Histologic preparations of joint and contiguous bone tissues after decalcification demonstrated marked exudate in the joint space, edema and hyperemia of the synovia, varying degrees of necrosis and repair of articular cartilage and focal necrosis of subchondral bone, as produced previously by septic arterial infarction of the femur. The bone abscesses beneath articular cartilage produced necrosis of the cartilage; those in the metaphyses spread along the epiphyseal cartilage plates, partially separated the epiphysis from the diaphysis and entered the joint near the attachment of its capsule. This association of a subchondral abscess with suppurative arthritis was demonstrated in all tissues examined. The septic infarcts were present also beneath many articular and epiphyseal cartilages without arthritis and beneath cartilage not contiguous with joints. It seems very probable that the peculiar structure and blood supply of growing bones and the susceptibility of these to infarction are important in the production of acute exudative arthritis. This is substantiated clinically by the great predominance of exudative arthritis in children over adults. The organism used was in no sense specific and similar changes were produced by a staphylococcus obtained from a cutaneous carbuncle.

8811 P

Ultrafiltration in the Concentration and Purification of Pneumococcus Specific Polysaccharides.

Rachel Brown.

From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y.

In continuance of the chemical and immunological studies of the pneumococcus,[1,2] methods of ultrafiltration previously described for use in the purification and concentration of other products[3,4] have proved distinctly advantageous in the preparation of the type-specific carbohydrate from broth cultures. Simultaneous concentration and purification are accomplished and the injurious effects of heat and chemicals are avoided. In one instance a 16-hour culture of pneumococcus type VII grown in an infusion-free peptone broth was run through a Sharples supercentrifuge and the effluent ultrafiltered and washed on a 4% nitrocellulose (Parlodion) membrane at room temperature. The residue, which contained all of the specific polysaccharide, represented a 100-fold concentration of the original material; at the same time 98.26% of the nitrogen had been removed. Similar results in the concentration of the specific polysaccharide and the elimination of inert material have been obtained by the passage of from 10 to 12 liters of clear broth culture through a single nitrocellulose-coated alumina thimble (127 x 45 mm.).

When larger amounts are to be handled, an initial concentration with ammonium sulfate can conveniently be used, followed by ultrafiltration for purification. If the broth-culture effluent from the Sharples supercentrifuge is treated with the optimum amount of ammonium sulfate (dependent upon the type of pneumococcus), the major portion of the specific polysaccharide is found in the scum which forms. This scum, when dissolved in water to give approximately a 50-fold concentration of the original, can then be purified by ultrafiltration.

Several alcoholic precipitations of the ultrafiltered residues give, in excellent yield, a product comparable in purity to those obtained by other methods and of high serologic activity.

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[1] Wadsworth, Augustus, and Brown, Rachel, *J. Immunol.*, 1931, **21**, 245; *ibid.*, 1933, **24**, 349.

[2] Brown, Rachel, Proc. Soc. Exp. Biol. and Med., 1935, **32**, 859.

[3] Wadsworth, Augustus, and Quigley, J. J., *Am. J. Hyg.*, 1934, **20**, 225.

[4] Wadsworth, Augustus, and Wheeler, M. W., *J. Infect. Dis.*, 1934, **55**, 123.

These methods have been found applicable to the preparation of the specific polysaccharides of pneumococcus types I, II, III, V, VII and VIII.

8812 P

Fixation of Ascorbic Acid by Tissues.

H. C. HOU.

From the Division of Physiological Sciences, Henry Lester Institute of Medical Research, Shanghai.

During the last few years considerable data have been accumulated to show that scorbutic animals suffer a marked depletion of the ascorbic acid content of the tissues particularly in organs such as the adrenal, pituitary, intestine, and liver. This depletion is readily made up when vitamin C rich foods are fed to the animals. The importance of the presence of ascorbic acid has been subsequently linked up with its function as an oxygen carrier, particularly through the studies of Harrison[1] and Euler and Klussmann.[2] They showed that tissue slices from scorbutic animals have a low oxygen uptake which is restored by the addition of ascorbic acid. Söderström and Törnblom[3] further show that in scorbutic animals the oxygen consumption is diminished.

The ability of tissues to fix ascorbic acid was suggested by the report of Harris, Ray and Ward.[4]. They believed from their study of the urinary output of vitamin C that the amount excreted might serve as a gauge of the nutritional state of the individual. This suggests that the tissues must be saturated with ascorbic acid before it will overflow through the kidney. This conception is supported by further observations of Harris and Ray,[5] Johnson and Zilva,[6] Ippen,[7] Euler and Malmberg,[8] Hou[9] and others on the urinary excretion of ascorbic acid in man and animals, in various nutritional states and following the administration of ascorbic acid orally or intravenously. Zilva[10] found a marked increase in the ascorbic acid content of the intestine of scorbutic animals after the injection of ascorbic acid. He[11] further showed that the antiscorbutic activity of the ascorbic acid and its related compounds appeared to be "connected with their capacity of being retained by the tissues of the animal organism." By means of histological staining with $AgNO_3$ Giroud and coworkers[12] were able to trace the deposition of ascorbic acid in the intestine, the blood, the liver and kidney, after orange juice was given to scorbutic animals. De Caro[13] studied the ascorbic acid content of the organs of normal and scorbutic guinea pigs at different periods following the intravenous injection of ascorbic acid and found a transient rise in normal animals. The concentration in the kidney and muscles of the scorbutic animals reached normal or even above normal in 15 minutes; other organs showed an increase although they did not reach a normal value. He further studied the effect of ascorbic acid solutions in contact with tissue emulsions and concluded that the behavior of the normal and scorbutic tissue emulsions was essentially the same. The slight difference obtained was considered to be due to loss in filtration and to the amount of ascorbic acid present in the tissue. De Caro thus believed that in scorbutic animals the ability of the tissue to fix ascorbic acid was decreased.

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[1] Harrison, D. C., *Biochem. J.*, 1933, **27**, 1501.

[2] Euler, H. v., and Klussmann, E., *Arkiv. Kemi, Mineral. Geol.*, B 11, No. 4, 1932-33.

[3] Söderström, N., and Törnblom, N., *Skan. Arch. Physiol.*, 1933, **66**, 67.

[4] Harris, L. J., Ray, S. N., and Ward, A., *Biochem. J.*, 1933, **27**, 2011.

[5] Harris, L. J., and Ray, S. N., *Lancet*, 1935, **228**, 71.

[6] Johnson, S. W., and Zilva, S. S., *Biochem. J.*, 1934, **28**, 1393.

[7] Ippen, F., *Schwei. Med. Wochenschr.*, 1935, **65**, 431.

[8] Euler, H. v. and Malmberg, M., *Svensk. Kem. Tidskr.*, 1935, **47**, 25.

[9] Hou, H. C., unpublished data.

We have recently investigated this problem in a slightly different manner and obtained results which indicate an increased capacity of the scorbutic tissues to remove ascorbic acid from solution.

Two groups of comparable guinea pigs were used. One group was kept on a vitamin C free diet (Hou[14]) and the other on a diet supplemented with plenty of cabbage. After 22 days the animals were killed by decapitation and bleeding. Tissues were removed at once, weighed, cut into slices, teased, put into Ringer's solution containing known (titrated) amounts of ascorbic acid and thoroughly mixed. The solutions were overlaid with liquid paraffin to prevent oxidation of the ascorbic acid by air and incubated at 37°. At the end of one hour and 5 hours, samples of one cubic centimeter of the solution were removed for titration with 2.6 dichlor-indophenol. The results obtained were compared with that of a control solution containing no tissue but similarly treated. It was found that the scorbutic tissues were able to take up more ascorbic acid from Ringer solution than the normal tissues. Among the tissues examined, adrenal and muscle took up most, next the skin, then the intestine and kidney. It is interesting to note that the adrenal took up to its full capacity during the first hour of incubation since nothing more was taken up during the next 4 hours of incubation. On the other hand, the intestine continued to take up more during the following four hours than during the first hour. The other tissues took up comparatively little during the second period.

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[10] Zilva, S. S., *Biochem. J.*, 1935, **29**, 100.

[11] Zilva, S. S., *Biochem. J.*, 1935, **29**, 1612.

[12] Giroud, A., Leblond, C. P., and Galelovitch, S., *C. R. Assoc. Anat. Bruxelles*, 1934, Mar., p. 4.

[13] De Caro, L., *H. S. Zeits. Physiol. Chem.*, 1934, **223**, 229.

[14] Hou, H. C., *Chinese J. Physiol.*, 1935, **9**, 253.

8813 C

Effect of Small Doses of Oestrin upon Ovarian and Vaginal Cycles of Mature Rats.

LUCIUS L. BUTTON AND CHARLES I. MILLER. (Introduced by G. W. Corner.)

From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

Much work has been done to demonstrate the effect of large doses of oestrin upon the ovaries, but scarcely any to show the effects of threshold or subthreshold doses. Bialet-Laprida,[1] injecting rats with 2-25 r.u. (Coward-Burn units) daily for 30 days, showed that the oestrous cycle was interrupted and also that the ovaries contained many atretic follicles at the end of this period. Thirty days after the injections were stopped the ovaries were slightly degenerate, there were few corpora lutea, many atretic follicles and the average weight of the ovaries was decreased. However there were also a few mature follicles present. Sixty days after the injections had ceased the ovaries were normal.

Selye, Collip and Thomson[2] treated 6 adult rats with 20 gamma of oestrin (200 int.u.) daily and obtained almost continuous vaginal oestrus. At the end of 20 days, however, the ovaries were noticeably atrophic, the average weight being 25 mg. In only one case did they contain recent corpora lutea.

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[1] Bialet-Laprida, Z., *Compt. Rend. Soc. Biol.*, 1934, **117**, 456.

[2] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1377.

De Castillo and Calatroni[3] injected mature female rats with 0.1 to 0.25 Coward-Burn rat units and found that after a latent period the oestrous cycles gradually became prolonged up to 40 to 80 days and eventually returned to normal length. At autopsy the ovaries contained no mature follicles and no recent corpora lutea.

Wade and Doisy[4] injected .65 to 13 gamma of oestrin (6.5-130 int.u.) daily for 60 days. The vaginal smears during this period showed prolonged periods of oestrus or dioestrus varying from 5 to 25 days interspaced with regular cycles.

In the present experiment young mature female rats were observed by the method of vaginal smears through a control period of 3 oestrous cycles, and were then treated with oestrin in the form of Progynon-B, kindly donated by the Schering Corporation. The hormone as diluted with sesame oil for use in these experiments was retested on castrated rats to insure correct potency. As controls, 10 rats were injected daily for 8 weeks with amounts of sesame oil equal to those used in the experimental injections. The ovaries of these rats showed no significant changes in appearance or weight and the vaginal cycles remained normal.

Groups of rats were injected subcutaneously with daily doses of .25, .50, 1.0, and 2.0 r.u. (approximately 1.25, 2.5, 5.0, and 10 int.u.) respectively for periods from 3 to 13 weeks. Another group of rats were used as untreated controls. Vaginal smears were made daily. At the end of the injection period the animals were killed, and the ovaries were dissected out, weighed, and examined under the binocular microscope. Serial sections of each ovary were made and the corpora lutea counted. The effects of the injections upon ovulation were determined by a comparison of the number of corpora lutea in the normal and experimental rats.

Effect on the cycle: Of the group of 5 rats receiving .25 r.u. per day, 3 exhibited no significant disturbance of cycles, and underwent a practically constant succession of oestrous cycles during the experimental period of ½ to 3 months. The remaining 2 exhibited some disturbance. In No. 26, for example, there were 4 regular cycles, followed successively by anoestrus for 10 days, 5 approximately normal cycles, another 10-day period of anoestrus and again 5 normal cycles. In No. 29 the cycles occurred with practically normal rhythm for 2½ months, after which there were 20 days of anoestrus (about 4 cycles omitted) followed by 2 oestrous smears 2 days apart, after which the animal was killed. At autopsy an ovarian tumor was found, which may possibly have been concerned with the slight irregularity of the cycle.

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[3] De Castillo, E.-B., and Calatroni, C.-J., *Compt. Rend. Soc. Biol.*, 1930, **104**, 1024.

[4] Wade, N. J., and Doisy, E. A., *Endocrinol.*, 1935, **19**, 77.

Another group of 7 rats received 0.5 r.u. daily for 3 months except that one rat (No. 13) was killed after 6 weeks. The effect on the vaginal cycle was variable. One rat (No. 13) exhibited normal cycles throughout the period of injection. In general, the others of the group had their cycles interrupted and periods of anoestrus varying in length from a few days (one cycle omitted or delayed) to as much as a month. These anoestrous periods were in several cases followed by periods of normal rhythm. Finally, one animal of this group (No. 3) after 5 approximately normal cycles had 4 oestrous periods at intervals of about 2 days, and then remained in continuous oestrus for 10 days, until autopsy.

A 3rd group of 3 rats received 1 r.u. per day for 2 to 7 weeks. The cycles were interrupted almost immediately. No. 19 went into anoestrus after one cycle and remained in the anoestrous state for 15 days, until killed. No. 2 after one cycle went into anoestrus for 2 weeks, then had 4 cycles and became anoestrous for 15 days until autopsy. Rat No. 10 went into continuous oestrus after 2 cycles. The fourth and last group received 2 r.u. daily. All these went at once, or after one cycle, into continuous anoestrus which lasted from 10 to 22 days. One was killed in this state; the other 2 went into continuous oestrus, in one case for 9 days, and in the other for 2 periods of 7 days each, interrupted by one day of dioestrus. After this phase of continuous oestrus both the animals resumed cycles of rather rapid rhythm (intervals of about 2 days).

In summary, the effect of daily treatment with oestrin in the form of Progynon-B in doses of 0.5 to 2.0 r.u. was to produce irregularities of the vaginal cycles, chiefly characterized by cessation of the rhythm in the anoestrous phase, and sometimes by continuous oestrus. The time of onset of the anoestrous state varied roughly with the doses; the larger dosages (1 r.u. and 2 r.u. daily) produced anoestrus at once or after one cycle, while the 0.5 r.u. dosage permitted several cycles before the supervening of the anoestrous state. With the dose of 0.25 r.u. daily 3 of 5 rats showed no disturbance of the vaginal cycle.

Effect on the ovaries: In the group of rats receiving 0.25 r.u. daily the ovaries were found to weigh less than those of the normal controls and also in every case the number of corpora lutea was found to be reduced. Whereas the ovaries of the normal rats weighed 40 to 45 mg., those of the experimental animals weighed only from 13 to 32 mg. Similarly, the control ovaries contained 75 corpora lutea while those of the experimental animals contained only ¼ to ½ this number. For example, the ovaries of rat No. 28 contained 47 corpora lutea, which is a definite reduction, and the ovaries of rats 6 and 26 contained only 19 and 16 corpora lutea respectively, which is a rather striking reduction.

In the group receiving 0.5 r.u. daily there was a considerable reduction in the corpora lutea in all cases except 2 in which there was still, however, a slight reduction. These 2 (Nos. 13 and 32) contained 57 and 62 corpora lutea, as compared to the others in this group which contained 14 to 37 corpora lutea, as shown by rats Nos. 24 and 30. The ovaries of rat No. 3 contained only 1 corpus luteum.

Approximately the same effects were obtained in the 2 groups of 3 rats each receiving 1 and 2 r.u. daily. There was a large reduction of the corpora lutea in all cases, with the numbers of corpora lutea present varying from 3 to 24. The weight of the ovaries was also reduced, varying from 9 to 30 mg., and seemed to vary directly with the number of corpora lutea.

In some of the animals which were autopsied at the end of long periods of induced anoestrous smears, large recent-looking corpora lutea were found. Whether this may be taken to indicate ovulation without corresponding vaginal changes, or rather indicates the persistence of corpora lutea under the influence of the oestrin treatment, cannot definitely be answered. We think it probable, however, that the latter alternative is correct, and that the effect of the treatment is actually suppression of ovulation with persistence of old corpora lutea, because (1) in some of the rats the number of corpora became very small, and only old retrogressing corpora remained; (2) Corner[5] found that proportionately comparable doses of oestrin caused suppression of ovulation in rhesus monkeys; (3) in a forthcoming note from this laboratory by Allen and Heckel, it will be shown that in rabbits the life of corpora lutea is prolonged by small doses of oestrin.

There is even evidence from the state of the ovaries that in some of our animals exhibiting regular vaginal cycles up to the time of autopsy ovulation was nearly or quite in abeyance.

These effects of the ovarian hormone upon the ovary itself are probably explainable as due to depression of the gonad-stimulating power of the hypophysis.

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[5] Corner, G. W., *Am. J. Physiol.*, 1935, **113**, 238.

It is evident from these results that the reproductive cycle cannot be determined accurately by means of the vaginal smear during periods of hormone treatment if small amounts of oestrin are present in the extracts administered. This must be taken into account, for example, in evaluating the results of those who have at various times attempted to inhibit the vaginal cycle with corpus luteum extracts, which when not fully purified contain small amounts of oestrin.

Conclusions: (1) Daily treatment with oestrin (Progynon-B) in very small doses (¼ to 2 r.u.) disturbs the vaginal cycle by inducing periods of continued anoestrous smears, or sometimes continued oestrous smears. (2) The ovarian cycle is also disturbed, the incidence of ovulation being reduced; and the weight of the ovaries is reduced.

We wish to express our appreciation to Dr. George W. Corner for his many helpful suggestions and criticisms.

8814 P

Migration of Nuclei in Uterine Epithelium of Monkey Following Prolonged Estrin Injections.*

MILTON D. OVERHOLSER AND WARREN O. NELSON.
From the Department of Anatomy, University of Missouri.

Eight *Macacus rhesus* monkeys received subcutaneous injections of estrin in oil solution (Amniotin† or Theelin‡) over time periods ranging from 6 to 24½ months. The total estrin dosage ranged from 36,400 to 73,400 rat units. Two of the animals were ovariectomized, 5 were normal, and 1 was depancreatized. Two ovariectomized animals were used as uninjected controls.

The migration of nuclei in the cells of the uterine epithelium (Fig. 1) occurred in 6 of the 8 injected animals. Of these, 3 were normal, 2 were ovariectomized, and 1 was depancreatized. The 2 negative cases (animals 14 and 59) were animals receiving the lowest dosages (36,400 and 44,150 r.u.). The ovariectomized controls were also negative. The nuclear migration is brought

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[*] Aided by a grant from the Committee on Scientific Research of the American Medical Association to Dr. M. D. Overholser.

[†] Supplied by Dr. J. A. Morrell of E. R. Squibb and Sons.

[‡] Supplied by Dr. Oliver Kamm of Parke, Davis and Co.

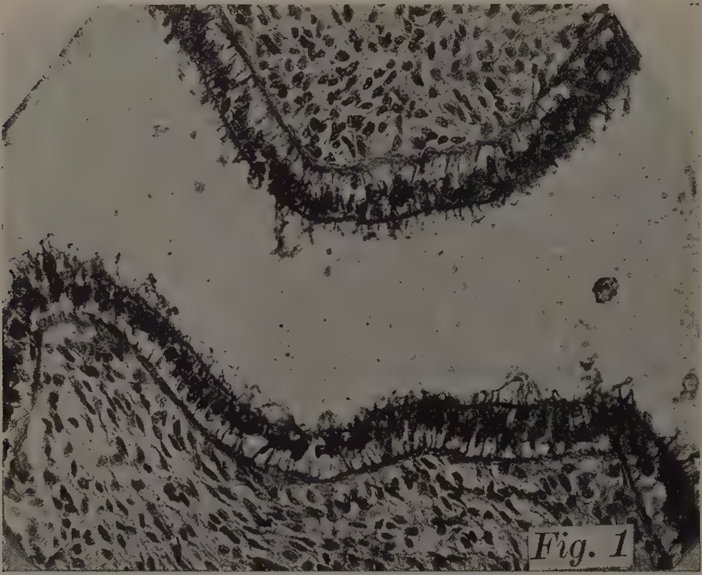

FIG. 1.

Uterine epithelium from ovariectomized animal 22, after receiving 300 R.U. of estrin daily over a period of 6 months. Total dosage 54,600 R.U. Migration of nuclei in epithelium. x 300.

about by the appearance of secretion at the bases of the epithelial cells. This causes the nuclei of the cells lining the lumina of the uterine cavity and glands alike, to be pressed toward the apices of the cells. Hisaw[1] (1935) and Lendrum and Hisaw[2] (1936) have noted this phenomenon as occurring regularly in the cells of the uterine epithelium of the monkey following injections of corporin, and have shown that it is due to an accumulation of glycogen beneath the nuclei. They state that the presence of glycogen is a characteristic feature of the endometrial response to the administration of corporin, but that minute traces of glycogen may be found occasionally in the basal ends of some cells following prolonged treatment with large doses of estrin. In our material the phenomenon occurred in ovariectomized and normal animals alike following the administration of estrin only. Our total estrin dosages were much greater than those of Lendrum and Hisaw. In other respects the endometrium showed a simple estrin response, the glands being straight with no cystic or "swiss cheese" effects. No stratified squamous cells were associated with the columnar uterine cells, although, with the exception of animals 14, 59 and 5 marked epidermization occurred in the cervix of the injected animals.

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[1] Hisaw, F. L., *Am. J. Ob. and Gyn.*, 1935, **29**, 638.

[2] Lendrum, F. C., and Hisaw, F. L., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 394.

8815 C

Studies of Prolactin in the Fowl Pituitary. I. Broody Hens Compared With Laying Hens and Males.

WILLIAM H. BURROWS AND THEODORE C. BYERLY.
From the National Agricultural Research Center, Beltsville, Maryland.

Riddle, Bates, and Lahr[1] have shown that the injection of prolactin into the fowl will produce broodiness. It seems to be a logical assumption that the pituitaries of broody hens would have a higher prolactin content than the pituitaries of laying hens or males. The present investigation was undertaken to ascertain if these differences in prolactin content of fowl pituitaries actually exist, and, if so, what their magnitude might be.

The pituitaries of broody hens, laying hens and males were implanted over the crop glands of 8- to 10-weeks-old White King pigeons (age computed from time of conception) according to the method of W. R. Lyons as described by McQueen-Williams[2] and the extent of local stimulation of the crop gland was accepted as a relative measure of the prolactin content of the pituitaries.

In all instances the pituitaries were implanted in pairs, *i. e.*, the pituitaries of two birds, either a broody hen and a laying hen or a broody hen and a male, were implanted into one pigeon; one over the right crop gland and one over the left crop gland.

In all but one of the pairs of implants in which a broody hen and a laying hen were represented, the reaction indicated that there was more prolactin in the pituitary of the broody hen than in that of the laying hen. The reactions were observed and graded with plus signs; 2 pluses being given where the reaction was regarded as being

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[1] Riddle, O., Bates, Robert W., and Lahr, Earnest L., *Am. J. Physiol.*, 1935, **3**, 352.

[2] McQueen-Williams, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 406.

TABLE I.

The prolactin-like reaction on the pigeon crop gland as shown by pairs of pituitaries implanted from broody hens and laying hens, and from broody hens and males.

Pigeon No.		Pituitary from broody hen No. of pluses	Pituitary from laying hen No. of pluses
4895		4	2
4281*		3	0
46†		4	3
22		5	2
67		5	4
19		6	3
71		3	1
16		2	1
13		3	0
10†		5	2
32		3	0
4		3	1
4675*		0	0
	Aver.	3.54	1.46
			Pituitary from male
4709†		7	0
6808†		7	0
6616†		6	0
4142†		5	0
2776†		5	3
4566†		5	3
	Aver.	5.83	1.00
4555†		4	
6077*		0	

*Broody hens that were nearly over their broody period.

†Broody hens that were just becoming broody.

twice that of a reaction designated by 1 plus, 3 pluses where the reaction was regarded as being three times as great as that of a reaction designated by 1 plus, etc. See Fig. 1.

In 13 cases of implants from broody hens paired with laying hens the average number of pluses recorded for the pituitaries of the broody hens is 3.54 and for the pituitaries of laying hens 1.46. The average number of pluses recorded for pairs of broody hens and males are 5.83 for the broody hens and 1.00 for the males.

Some of the broody hens were selected at the first sign of broodiness, others were at some indefinite stage of broodiness. Three hens whose pituitaries were used in pigeons Nos. 4281, 4675, and 6077 were found to be almost at the end of their broody period and this may account for the faint reaction obtained from one of them and the negative results from the other two.

Although it is obvious from the data in the table that the prolactin content of the pituitaries of broody hens is greater than that of the pituitaries of laying hens or males, the indication that there is more prolactin in the pituitary of a hen in early broodiness than in that

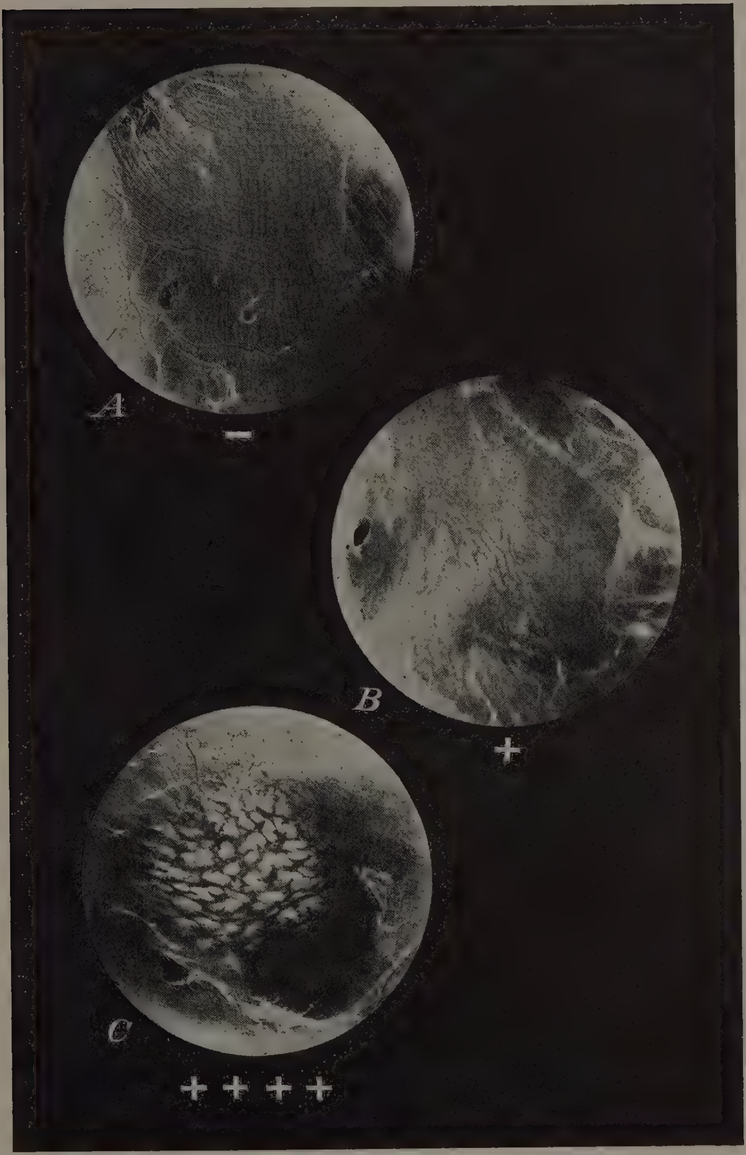

FIG. 1.

Typical reaction of pigeon crop glands to implanted fowl pituitaries. (A) Negative. (B) Slightest reaction classed as positive. (C) Considered as being 4 times greater reaction than that shown in B.

of one nearly over brooding can be only a surmise until more data are available.

Conclusions. It has been found that single fowl pituitaries implanted over the crop glands of 8- to 10-weeks-old pigeons (age computed from time of conception) will, in many instances, cause a prolactin-like reaction. It is assumed that this reaction is caused by prolactin in the pituitaries of the donating fowls.

Pituitaries from broody hens cause a greater reaction of the pigeon crop gland than the pituitaries of laying hens. Pituitaries from males cause a reaction about equal to, or slightly less, than the pituitaries from laying hens.

It was noted in a few cases that the pituitaries of hens just becoming broody gave a greater reaction than those of hens nearly over their broody period. More data are being gathered on this point.

8816 C

Studies of Prolactin in the Fowl Pituitary. II. Effects of Genetic Constitution With Respect to Broodiness on Prolactin Content.

T. C. BYERLY AND W. H. BURROWS.

From the National Agricultural Research Center, Beltsville, Maryland.

Broodiness was induced in laying fowls of broody races by the injection of prolactin, by Riddle, Bates, and Lahr.[1] After prolactin injections laying fowl of non-broody races and males of a broody race became only partially broody, usually clucking without nesting. Burrows and Byerly[2] showed that pituitaries from broody fowl have a much greater prolactin content than those from laying fowl and from males, as judged by local pigeon crop-gland response to pituitary implantation.

The present work was undertaken to determine the relative potency of fowl pituitaries with respect to genetic constitution of the fowls. White King pigeons of 8-10 weeks conception age were used as recipients for the fowl pituitaries. The local implantation technique of Lyons as described by McQueen-Williams[3] was used. The crop-gland responses were recorded as "plus" when any visible

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[1] Riddle, O., Bates, R. W., and Lahr, E. L., *Am. J. Physiol.*, 1935, **111**, 352.

[2] Burrows, W. H., and Byerly, T. C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 841.

[3] McQueen-Williams, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 406.

TABLE I.
Local Crop Gland Response to Implantation of Pituitaries from Birds of Proven or Presumptive Broody or Non-broody Genetic Constitution.

	Laying fowl				Mature males			
	Broody genetic constitution (proven)		Non-broody genetic constitution (presumptive)		Broody genetic constitution (presumptive)		Non-broody genetic constitution (presumptive)	
Donors	No. of donors	Aver. response	No. of donors	Aver. No. of pluses	No. of donors	Aver. No. of pluses	No. of donors	Aver. No. of pluses
Single Comb White Leghorn	*	*	7	0.6	*	*	11	1.1
Crossbred	21	3.0	14	1.5	10	1.5	*	*
Single Comb Rhode Island Red	*	*	*	*	*	*	4	1.0
Totals and averages	21	3.0	21	1.2	10	1.5	15	1.1

stimulation was obtained, "2 plus" when the stimulation was roughly twice the minimal visible response, and so on.

Crossbred females were used in their second laying year. Occurrence or absence of broodiness during their first laying year was assumed to reflect their genetic constitution with respect to broodiness. Crossbred males used were assumed to be genetically broody since females from the crosses showed a high incidence of broodiness. The crossbreds were chiefly from matings of Single Comb Rhode Island Reds x Barred Plymouth Rocks, and White Wyandottes x Single Comb White Leghorns. The Single Comb White Leghorn females and males and the Single Comb Rhode Island Red males used were assumed to be of non-broody genetic constitution, since incidence of broodiness is low in birds of these breeds in the flocks at the National Agricultural Research Center. Obviously, broody constitution of the females so designated is proven while the genetic constitution of the other groups is presumptive. Fortunately, presence of broody birds among the presumptively non-broody ones could only reduce, not increase, the apparent difference found between pituitaries from birds of broody and non-broody genetic constitution.

The data are summarized in Table I. They show clearly that pituitaries from laying hens of known broody constitution have greater crop-stimulating potency than those from laying birds of presumptive non-broody constitution. There were 9 paired experiments in which a pituitary from a laying hen of proven broody genetic constitution was implanted over one crop-gland and a pituitary from a laying hen of presumptive non-broody genetic constitution over the other. The response to the pituitary from the hen of broody genetic constitution was greater in 8 of the 9 cases and equal to that from the presumptively non-broody hen in one case.

The data for males indicate that the male pituitary is about equal in crop-stimulating potency to the pituitary of the genetically non-broody female. It is likely that males of broody genetic constitution have a higher prolactin content than those from males of non-broody genetic constitution but the difference in crop stimulation was so small that the data are too few to make this conclusion certain. Paired implants from males and females of proven and presumptive broody and non-broody genetic constitution, and vice versa, were in accord with the summarized data of Table I. This was also true of paired implants from males of presumptive broody and non-broody genetic constitution.

8817 C

Effect of Route of Administration on the Bioassay of Prolactin.*

ROBERT W. BATES AND OSCAR RIDDLE.
Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, L. I., N. Y.

Our published method[1] for the bioassay of prolactin is based on the growth response of the crop-glands of pigeons after injection into the pectoral muscles. The data of the present communication show that when prolactin is injected otherwise than by the intramuscular route very widely different values are obtained. This point has some immediate practical importance since intramuscular injection of pigeons is being used for this assay in several laboratories, and the results here reported indicate that injections so made as to permit seepage from the muscle into subcutaneous areas would yield erroneously high values. It is also of theoretical interest that mere mode of administration of this hormone may have as much as a 12-fold effect on its potency as a stimulator of the crop-gland.

In previous studies the great influence of age[1] and race of pigeon upon this bioassay was demonstrated. The present study was therefore made on pigeons of the same age and breed. White Carneaux (from the Palmetto Pigeon Plant, Sumter, S. C.) were injected and killed at 6 to 7 weeks after hatching. Five routes of injection were tested; intramuscularly, deep into the breast muscle; intravenously into the wing veins; subcutaneously, and intracutaneously over the breast muscle or on the back and sides behind the leg—the region of the crop-sacs being carefully avoided. The volume injected was 0.4-0.5 cc. daily excepting that 0.08 cc. was used intracutaneously. All birds were injected once daily for 4 days and killed 94-100 hours after the first injection. The same preparation (No. 469) of prolactin was used throughout.

The more essential data—those showing the relative potency of 1 mg. prolactin when administered by 5 different routes—are presented in Table I. These data show that the subcutaneous and intracutaneous routes are most effective; least effective is intravenous injection. The crop-sacs of all birds injected intravenously showed visible thickening when held against a source of light,

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* Aided by a grant from the Carnegie Corporation of New York.

[1] Riddle, O., Bates, R. W., and Dykshorn, S. W., *Am. J. Physiol.*, 1933, **105**, 191.

TABLE I.

Quantitative Response of Crop-glands of Immature Pigeons to Prolactin Administered by 5 Different Routes. Dosage = 1 mg. per day for 4 days.

Route of injection	No. birds	Body wt.	Crop-gland weight Av.	Crop-gland weight σ	Potency* Units/mg.
		gm.	mg.		
Control	30	476	1245	± 33	0.0
Intravenous	10	498	1610	± 113	1.0
Intraperitoneal	10	465	1890	± 91	1.4
Intramuscular	15	457	2355	± 188	2.4
Intracutaneous	10	460	3790	± 233	10.0
Subcutaneous	15	459	3940	± 232	11.5

*Calculated unitage per mg. corresponding to the average crop-gland weight.

although their weights were not all significantly increased; and a similar amount of stimulation was obtained in 10 birds to which 0.1 mg. was administered subcutaneously. This confirms the values of units per mg. as calculated from the average crop-gland weights and shown in the last column of the table.

The conversion of increased crop-gland weights into prolactin units makes possible the expression of the efficiency of different routes of administration in simple terms. Subcutaneous injection is thus found to be about 5 times as efficient as intramuscular and about 11.5 times as efficient as intravenous injection. These differences in response are perhaps associated mainly with unequal rates of absorption from the sites of injection; but it is also possible that unequal rates of enzymatic destruction of hormone at the various sites of administration are involved. Bearing on this point is our earlier reported[1] result showing that dividing the daily (intramuscular) doses of prolactin into three spaced injections gives an increased effect on the crop-gland. Since that increase is of the order of four-fold one observes that the division of an intramuscular dose into more than 3 time-spaced injections should make it as effective as the same amount of prolactin given in a single subcutaneous injection.

We have confirmed the finding of Lyons and Page[2] that the amount of prolactin that can be detected by single intracutaneous (intradermal) injection directly over the crop-sac (10 tests) is about 1/100th that which can be detected by injection elsewhere in the body. This is not true, however, of single subcutaneous injections made directly over the crop-gland; for, in 23 such tests we found the threshold dose not greatly different from that of daily injections over a 4-day period into subcutaneous areas remote from the crop-sacs. Here again we find another striking example of the importance of route of injection. It is notable also that if subcutaneous instead of intramuscular injection were used in the "threshold" bioassay method of McShan and Turner[3] a unit of notably smaller size than that described by them would be expected, unless indeed seepage into the subcutaneous area normally occurs after their injections into very superficial parts of the pectoral muscles of the pigeon.

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[2] Lyons, W. R., and Page, Emery, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1049.

In our tests with the effect of route of administration on the potency of pituitary F.S.H. we have failed to obtain a significant difference (weights of testes of immature pigeons). And, again unlike prolactin, division of the daily dose of F.S.H. into 3 time-spaced injections failed to change the amount of the response of the testes. In the rat, however, Collip has found subcutaneous injection of gonadotropic hormone much more effective than intraperitoneal injection.

Summary. In young pigeons of the same age and race the response of the crop-glands to prolactin administered by 5 different routes has been studied and found to differ widely. Subcutaneous and intracutaneous injections are about 11 times as efficient as intravenous injections, about 5 times as efficient as intramuscular injections, and about 8 times as efficient as intraperitoneal injections at the dosage level used.

8818 C

Type-Specific Anti-M Precipitins in Rheumatic and Non-Rheumatic Patients With Hemolytic Streptococcal Infections.

HOMER F. SWIFT AND B. E. HODGE.

From the Hospital of the Rockefeller Institute for Medical Research, New York.

The thesis concerning the relationship between streptococcal infections, rheumatic fever, and rheumatoid arthritis, rests in part on clinical observation and in part on a study of antibodies against streptolysins,[1] fibrinolysins,[2] and various chemical components of hemolytic streptococci. The chief of these components in respect of immunological classification have been described by Lancefield.[3] Under suitable conditions most group A hemolytic streptococci induce the formation of antistreptolysins, antifibrinolysins, anti-C precipitins, and anti-P precipitins; and these four antibodies have been demonstrated in the serum of patients with various streptococcal infections. Because they have also been found by some observers in a high proportion of patients with rheumatic fever,[1,4,5,6] it has been suggested that there is a close connection between streptococcal infection and this disease. The demonstration of agglutinins against hemolytic streptococci[7,8] and the existence of group A anti-C precipitins in the sera of patients with rheumatoid arthritis[9,6,10] has, furthermore, indicated that the etiology of this disease may rest in part, at least, upon a streptococcal basis.

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[3] McShan, W. H., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 50.

[1] Todd, E. W., *Brit. J. Exp. Path.*, 1932, **13**, 248.

[2] Tillett, W. S., *J. Bacteriol.*, 1935, **29**, 111.

None of these antibodies has a type-specific relationship; in other words, they may result from streptococcal infection with any member of group A of Lancefield. On the other hand, the protective action of an anti-streptococcal immune serum runs roughly parallel with its content of anti-M precipitin;[11] M appears to be the type-specific substance of group A hemolytic streptococci.[3] Until the present time, however, no reports have appeared dealing with the anti-M content of the serum of patients suffering from hemolytic streptococcal infection, nor of those with rheumatic fever.

This information is most desirable, for it seems probable that elimination of part of the pathogenic action of these microörganisms depends upon the development of a suitable degree of type-specific immunity.

This report deals with 2 groups of patients: (I) 24 suffering from streptococcal infections, without demonstrable rheumatic sequelæ; (II) 17 with streptococcal infections and rheumatic sequelæ. Group I consisted of 6 cases of otitis media, 4 of scarlet fever, 4 of tonsillitis, 5 of sore throat, and one of cervical adenitis.* The streptococci from 15 of these were obtained from throat-cultures; 6 were from pus from the middle ear, one from pleural exudate, one from a purulent lymph-node, and one from sputum. In group II the antecedent streptococcal infection was not always clear. There were 2 cases of otitis media, 2 of tonsillitis, 3 of sore throat, one of head cold, and in 8 the history of recent upper respiratory infection was negative. The streptococci in 6 patients were obtained from the throat, in one from the middle ear, and in 10 from the interior of excised tonsils. Throat cultures from these 10 patients yielded too few hemolytic streptococci to be of significance.

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[3] Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 91, 469, 481, 843, 857.

[4] Coburn, A. F., and Pauli, R. H., *J. Exp. Med.*, 1932, **56**, 651.

[5] Coburn, A. F., and Pauli, R. H., *J. Clin. Invest.*, 1935, **14**, 769.

[6] McEwen, C., Bunim, J. J., and Alexander, R. C., *J. Lab. and Clin. Med.*, 1936, **21**, 465.

[7] Cecil, R. L., Nicholls, E. E., and Stainsby, W. J., *Am. J. Med. Sc.*, 1931, **181**, 12.

[8] Dawson, M. H., Olmstead, M., and Boots, R. H., *J. Immunol.*, 1932, **23**, 187.

[9] Dawson, M. H., Olmstead, M., and Jost, E. L., *J. Immunol.*, 1934, **27**, 355.

[10] Dawson, M. H., Olmstead, M., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 80.

[11] Lancefield, R. C., and Todd, E. W., *J. Exp. Med.*, 1928, **48**, 769.

All the strains were shown by Dr. Lancefield to belong to group A; they grew in matt colonies.[12] The M precipitinogens were obtained from HCl extracts, which were neutralized and freed from group-specific carbohydrate, C, by 2 precipitations with alcohol.[13]

Sera were collected at irregular intervals from one week to 7 or 8 months following the onset of illness. The specimens from patients with uncomplicated streptococcal infections, as a rule, were obtained earlier than those from the rheumatic patients, for the latter were not usually seen until rheumatic symptoms had existed for a few days to a week or more. On the other hand, the rheumatic patients were sick longer; and hence specimens were obtained over a longer period. In addition to the test for homologous anti-M precipitins, tests were made to detect heterologous anti-M precipitins, anti-C, and anti-P precipitins, and anti-streptolysins. The titer of the latter ranged from 25 to 1,200 or 1,600 units per cc. of serum, with a median of 200 to 250 units; no significant difference was found between the rheumatic and non-rheumatic groups.

Table I gives the cumulative totals by weeks, of the various strengths of homologous anti-M precipitins developed by the two groups of patients. It is arranged so that any given degree of reaction is indicated in the same category as that in which it was originally detected until there was a change to another category. No attempt was made to continue the tests until the reactions became negative. This arrangement of the table permits a comparison of the time the stronger degrees of reaction appeared.

There is an obvious difference in the 2 groups. From relatively few + or ++ reactions in group I during the first 2 weeks the ratio shifted so that 70% were of this strength by the 4th week and over 80% by the 5th week.

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* We are indebted to Dr. Currier McEwen for the cultures and serum from some of the patients with tonsillitis; and to the Medical Board of Willard Parker Hospital for the privilege of studying the scarlatinal patients.

[12] Todd, E. W., *Brit. J. Exp. Path.*, 1928, **9**, 1.

[13] Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 91.

In group II the period indicated was dated from the onset of rheumatic symptoms rather than from the onset of streptococcal infection, as was done in group I. This was necessary because the patients were not seen until after rheumatic symptoms had developed. To make the timing in the 2 groups comparable, from 1 to 3 weeks should probably be added to each period charted in group II.

Four patients in group II had strong reactions when examined the second week. One of them had otitis media, one tonsillitis, and 2 rather severe sore throats. These 4 had relatively mild rheumatic fever, with persistently anti-M reactions. Among the remaining 13 patients, strong reactions did not appear until distinctly later than was the case in group I; and not until the 3rd month were over 80% included in the + and ++ categories. In some patients anti-M reactions did not become even + until the 4th or 5th month, at a time when recovery was taking place. Other patients with persistent ± reactions had prolonged low-grade rheumatic infection.

As the readings recorded were made after 48- or 72-hour standing in the ice-box in addition to 2-hour incubation at 37°, *and not after centrifugalization,* it is felt that the ++ reactions have real significance. Incidentally it may be noted that readings not recorded here were also made after centrifugalizations, and that they were usually stronger than those charted. When a reaction no stronger than + was detected it is possible that this reaction might have been due to some other antibody of unknown nature. Such a reading indicated, however, that the patient had not developed strong type-specific immune bodies. On the other hand, because the appearance and intensity of these reactions was not paralleled by the presence of other types of precipitins, we felt that they were due to specific anti-M antibodies. The full significance of their occurrence cannot be definitely determined with the data at hand. Their presence in non-rheumatic patients indicates that they were probably not responsible for rheumatic manifestations. In fact, the relatively mild rheumatic symptoms among rheumatic patients who early showed strong anti-M reactions, and the tendency, in some instances, towards recovery at the time of the appearance or increase in strength of the type-specific antibody, indicate that the presence of this antibody had a favorable implication.

TABLE I.

Anti-M Reactions in Patients with Hemolytic Streptococcal Infections.

Degree of anti-M reaction	Week→ 1 No.	2 No.	3 No.	4 No.	5 No.	6 No.	7 and 8 No.			
	Period after onset of streptococcal infection.									
Group I—Non-Rheumatic										
—	8 = 66%	5 = 25%	2 = 9%	1 = 4%	1 = 4%	1 = 4%	1 = 4%			
±	3 = 25%	10 = 50%	11 = 48%	6 = 25%	3 = 12%	3 = 12%	3 = 12%			
+	1 = 8%	3 = 15%	8 = 35%	9 = 38%	9 = 38%	8 = 33%	8 = 33%			
++ +++	....	2 = 10%	2 = 9%	8 = 33%	11 = 46%	12 = 50%	12 = 50%			
Total	12	20	23	24	24	24	24			
	Period after onset of rheumatic fever.									
Group II—Rheumatic	Week→ 1	2	3	4	5	6	7 and 8	Month→ 3	4	5
—	....	3 = 33%	4 = 33%	5 = 35%	5 = 35%	2 = 13%	1 = 6%	1 = 6%	3 = 17%	4 = 23%
±	....	1 = 11%	1 = 8%	1 = 7%	1 = 7%	4 = 27%	5 = 29%	2 = 13%	1 = 6%	1 = 6%
+	....	1 = 11%	3 = 25%	4 = 28%	3 = 21%	4 = 27%	6 = 35%	6 = 35%	5 = 29%	3 = 17%
++ +++	1	4 = 45%	4 = 33%	4 = 28%	5 = 35%	5 = 33%	5 = 29%	8 = 47%	8 = 47%	9 = 53%
Total	1	9	12	14	14	15	17	17	17	17

Summary. The development of type-specific anti-M precipitins was studied in 2 groups of patients suffering from hemolytic streptococcal infections. Most of the patients in group I, none of whom developed rheumatic fever, showed relatively strong type-specific reactions in their serum by the 4th or 5th week after the onset of infection. The members of group II all developed rheumatic fever; some had similar strong antibodies early; but most rheumatic patients from whom hemolytic streptococci were obtained in significant numbers did not show strong anti-M precipitins until distinctly later than was the rule for the non-rheumatic group.

8819 C

Rate of Urinary Excretion of Test Doses of Ascorbic Acid.

ESTELLE E. HAWLEY* AND DORAN J. STEPHENS.

From the Departments of Pediatrics and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

Studies of the urinary excretion of test doses of ascorbic acid have been concerned for the most part with the total amount excreted during the 24-hour period following the administration of the vitamin C preparation. A few isolated observations[1–5] have indicated that a large portion of the total amount excreted appears in the urine during the first few hours after the test dose is given. This report presents the results of a detailed study by the authors of the rate of excretion of test doses of ascorbic acid, administered by mouth and intravenously, under conditions of vitamin C depletion and saturation.

Test doses of 100 or 200 mg. of ascorbic acid were given in the form of orange juice or crystalline ascorbic acid† by mouth, and in the form of crystalline ascorbic acid intravenously, to subjects whose states of vitamin C saturation were known. For the most part, states of vitamin C depletion or "unsaturation" followed the intentional administration of a diet low in anti-scorbutic foods for several days. States of vitamin C saturation were induced by the administration of large amounts of orange juice until a large part of the daily test dose appeared in the 24-hour urine specimens. For the experiments here reported, urine specimens were collected at hourly intervals for six hours after the administration of the test dose, then in 2 3-hour periods. Urine voided between the 12th and 24th hours was pooled in a single 12-hour sample. Each specimen of urine was placed immediately after voiding in a dark brown, stoppered bottle containing glacial acetic acid. It was then placed in the ice-box until titrated. The ascorbic acid content was determined by titration against a standardized solution of 2:6 dichlorophenol-indophenol according to the method described by Birch, Harris and Ray.[6] In all instances, the titrations were carried out on the same day, usually within one hour after voiding.

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* Aided by a grant from the California Fruit Growers' Exchange.

[1] Harris, L. J., *Biochem. J.*, 1933, **27**, 2011.

[2] Johnson, S. W., and Zilva, S. S., *Biochem. J.*, 1934, **28**, 1393.

[3] Harris, L. J., and Ray, S. N., *Lancet*, 1935, **1**, 71.

[4] Schroeder, H., *Klin. Wchnschr.*, 1935, **14**, 484.

[5] Hawley, E. E., Stephens, D. J., and Anderson, G. K., *J. Nutrition*, 1936, **11**, 135.

† We are indebted to Merck & Co. for the crystalline ascorbic acid ("Cebione") used in these experiments.

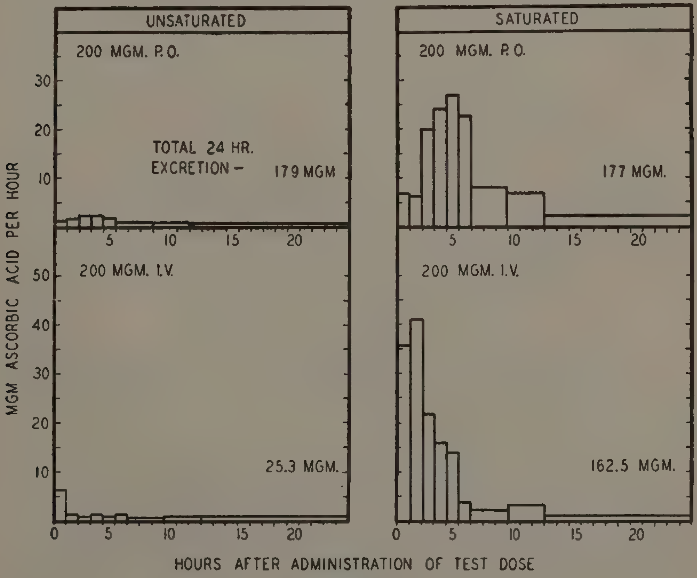

FIG. 1.

Hourly excretion of ascorbic acid after four comparable test doses given to subject L.B.

Fig. 1 presents typical urinary responses to 4 comparable test doses given to a single individual and shows in graphic form the hourly excretion of ascorbic acid given orally and intravenously, in the unsaturated state and after saturation with orange juice. The results of 50 such experiments in unsaturated and saturated subjects are summarized in Fig. 2 which shows the composite curves of the cumulative hourly excretion of test doses of ascorbic acid expressed as % of the test dose which was excreted and as % of the total 24-hour excretion.

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[6] Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 590.

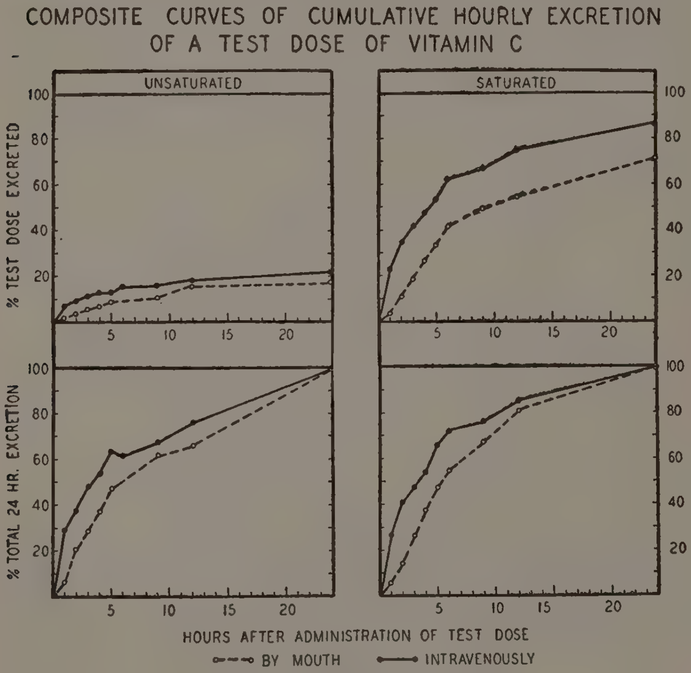

FIG. 2.

Summary of 50 experiments, showing the rate of excretion of test doses of ascorbic acid administered by mouth and intravenously to saturated and unsaturated subjects.

It has been repeatedly shown that the administration of a large test dose of ascorbic acid to a subject whose stores have been depleted of vitamin C results in the excretion of an amount of ascorbic acid but little, if any, larger than that excreted during a control period without the test dose. In such subjects only a very slight increase in the rate of excretion occurred during the first few hours after the test dose was given (Fig. 2). After intravenous administration, the rate of excretion was only slightly greater than when the vitamin C was given by mouth. Even after the introduction of the ascorbic acid directly into the blood stream it was apparently removed from the circulation so rapidly that only a very small portion of the test dose found its way into the urine.

In the subjects who had been taking large amounts of orange juice, from 40 to more than 100% of the test dose appeared in the urine within 24 hours. These individuals excreted a large portion of the ascorbic acid within the first few hours after it was given. After intravenous administration, maximum excretion occurred during the 1st and 2nd hours. When the ascorbic acid was given by mouth, maximum excretion occurred during the 3rd, 4th, 5th, and 6th hours after administration. Delay occasioned by the absorption of the ascorbic acid from the gastro-intestinal tract was apparently responsible for the differences in the rate of excretion observed after oral and parenteral administration.

The composite curves of cumulative hourly excretion calculated as % of the total 24-hour excretion are of particular interest. When the test dose was given orally to subjects who had been partially depleted of vitamin C, approximately 47% of the total 24-hour excretion appeared in the urine by the end of the 6th hour, 67% by the end of the 12th hour. When the test dose was given intravenously to similar subjects, 60% of the total 24-hour excretion appeared in the first 6 hours, 75% in 12 hours. In the saturated subjects, 40% of the total amount excreted in 24 hours appeared in the urine within 2 hours after intravenous administration of the test dose, 72% by the end of the 6th hour and 85% by the end of the 12th hour. When the test dose was given by mouth, the corresponding percentages for the 2nd, 6th and 12th hours were 14, 55 and 81 respectively. It is apparent that the first 12 hours after the administration of a test dose of ascorbic acid are the most important from the point of view of urinary excretion, regardless of the state of saturation or the method of administration of the test dose.

Summary. The rate of excretion of ascorbic acid was determined after the oral and intravenous administration of test doses to unsaturated and saturated subjects. In those subjects whose reserves of vitamin C had been depleted, there was only a slight increase in the rate of excretion during the first few hours, regardless of the method of administration. In saturated subjects, an

average of 80 to 85% of the total 24-hour excretion occurred during the first 12 hours after administration of the test dose. When the latter was given intravenously, maximum excretion occurred during the 1st and 2nd hours. After oral administration, maximum excretion occurred during the 3rd, 4th, 5th, and 6th hours.

8820 C

Properties of Different Fibrinolysins Produced by Streptococci.

E. Witebsky and E. Neter.

From the Bacteriological and Serological Laboratories of the Beth Israel Hospital, New York City.

Tillett and Garner[1] reported that hemolytic streptococci and some strains of staphylococci produce a fibrinolytic substance; this observation was corroborated by Schmidt.[2] But, Tunnicliff[3] found some strains of *S. viridans* to be fibrinolytic, and Madison[4] reported fibrinolysin-production by *B. pestis*. In a previous paper[5] the production of fibrinolysin by *S. hemolyticus* and *viridans, enterococcus, pneumococcus* and others was described. The discrepancy in these results may be explained by differences in the culture media employed: When 0.05% glucose-broth or 5% serum-broth was used, hemolytic streptococci only produced the fibrinolysin; when cultivated in 2% glucose-broth, however, various kinds of microörganisms proved to be fibrinolytic. The experiments to be reported are concerned with the question whether the fibrinolysins obtained in these media have different properties.

Technic. Infusion broths, containing 1% peptone, 0.05% and 2% glucose, respectively, with pH 7.4, were employed. Strains from human sources were incubated for 18 hours at 37° C. The supernatant fluid was tested for fibrinolytic activity according to the usual technic.[1]

Plasma was obtained by mixing 10 cc. of normal human blood with 1 cc. of 2% solution of potassium oxalate; it was stored in refrigeration not longer than 48 hours; supernatant fluid of a cul-

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[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Schmidt, H., *Zeitschrift für Immunitaetsforschung*, 1936, **87**, 1.

[3] Tunnicliff, R., *J. Infect. Dis.*, 1936, **58**, 92.

[4] Madison, R. R., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 301.

[5] Neter, E., and Witebsky, E., Proc. Soc. Exp. Biol. and Med., 1936, **34**, 549.

TABLE I.

Quantitative Titration of Fibrinolysins of *S. Hemolyticus.*

Decreasing amounts of supernatant fluid (volume 0.5 cc.)	Strain 693 in 2% glucose-broth				Strain 2327 in 0.05% glucose-broth			
	a	b	c	d	a	b	c	d
1. 0.5	++++	++++	++++	++++	—	++	++++	++++
2. 0.166	—	—	—	—	—	++++	++++	++++
3. 0.055	—	—	—	—	—	++++	++++	++++
4. 0.019	—	—	—	—	—	++	++++	++++
5. 0.006	—	—	—	—	—	—	+++	++++
6. 0.002	—	—	—	—	—	—	—	++++
7. 0	—	—	—	—	—	—	—	—

Read after a—10 minutes 37° C.
b—40 ” ”
c—90 ” ”
d—24 hours ”
+ - ++++ = various degrees of fibrinolysis.
— = no fibrinolysis (coagulation).

ture (0.5 cc.) was mixed with 1 cc. of 1:5 dilution of human plasma; then 0.25 cc. of a 0.25% solution of calcium chloride in normal saline was added. The mixtures were shaken thoroughly, kept at 37° C., and the results read at various intervals.

Results. In the first experiment, 2 different strains of *S. hemolyticus,* one producing the fibrinolysin in 2% glucose-broth* but not in 0.05% glucose-broth, and the other in 0.05% glucose-broth, were incubated in 2% glucose-broth and 0.05% glucose-broth, respectively, for 18 hours at 37° C. The supernatant fluids of these cultures, in decreasing amounts (volume 0.5 cc.), were tested for fibrinolytic activity.

Table I shows that the 2% glucose-broth culture of strain 693 is fibrinolytic only when used undiluted, and that a dilution of 1:3 is ineffective; the 0.05% glucose-broth of strain 2327 is fibrinolytic up to a dilution of 1:81 after 90 minutes, and up to 1:243 after 24 hours. Further, strain 693, in 2% glucose-broth, inhibits the coagulation of plasma constantly, while with strain 2327, in 0.05% glucose-broth, clotting of the plasma occurs first, and lysis then follows.

Many strains of hemolytic streptococci produce fibrinolysin in 0.05% as well as in 2% glucose-broth. When such strains were tested for fibrinolytic activity, it was found that both produce potent fibrinolysin and cause secondary lysis of previously clotted plasma. The fibrinolysin produced by such strains in both media belongs to the 0.05% glucose-broth type.

Because Tillett[6] found that the fibrinolysin produced by hemolytic streptococci of human origin in 0.05% glucose-broth is effective toward human plasma only, it was of interest to determine whether the specificity for human plasma also applied to the 2% glucose-broth fibrinolysin. For this purpose, 2 strains of hemolytic streptococci—one (693) producing fibrinolysin in 2% glucose-broth only, the other (1755) in 0.05% glucose-broth—were incubated in the respective glucose-broths for 18 hours at 37° C. The supernatant fluid of these cultures was tested for fibrinolytic activity toward human as well as rabbit and guinea-pig plasma. Strain 693, in 2% glucose-broth, inhibited the coagulation of human, rabbit, and guinea-pig plasma. Strain 1755 in 0.05% glucose-broth dissolved human but not rabbit or guinea-pig clot. Strains of hemolytic streptococci capable of producing fibrinolysin in 2% as well as in the 0.05% glucose-broth were found to be lytic for human plasma clots only.

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* The 2% glucose-broth was used as representative of broths with higher concentrations of glucose; as reported previously,[5] it is possible to reduce the glucose-concentration to 0.4% and still obtain fibrinolysin from these strains.

[6] Tillett, W. S., *J. Bact.*, 1935, **29**, 111.

The fibrinolysin produced in 2% glucose-broth only, is not altered by being heated at 60° C. for an hour. The fibrinolysin of the 0.05% glucose-broth is also heat-stabile; this corroborates the findings of Garner and Tillett.[7] On the other hand, according to Schmidt,[3] the fibrinolysin obtained in 5% horse-serum broth is almost completely destroyed by this procedure. These results suggest the existence of even more than 2 types of fibrinolytic agents of bacterial origin.

Tillett and Garner,[1] Van Deventer,[8] and Schmidt[2] demonstrated that the fibrinolysins produced by hemolytic streptococci in 0.05% glucose-broth and 5% horse-serum broth may be neutralized by certain streptococcal antisera. Experiments were therefore undertaken to ascertain the influence of streptococcal antiserum upon the 2% glucose-broth fibrinolysin of hemolytic streptococci.

Two different strains of *S. hemolyticus*—one producing the fibrinolysin in 2% glucose-broth only, the other in 0.05% glucose-broth—were cultivated in the respective glucose-broths. A preliminary titration was performed with the supernatant fluids. In this experiment the 2% glucose-broth culture was used undiluted, the 0.05% in a dilution of 1:10. Both broths (volume 0.5 cc.) were mixed with decreasing amounts (0.5 cc.) of normal human spinal fluid, normal horse-serum and streptococcal antiserum;† the mixtures were incubated for an hour at 37° C. Then 1cc. of a 1:5 dilution of human plasma and 0.25 cc. of 0.25% calcium-chloride solution were added. The results are given in Table II.

Table II shows that the effectiveness of fibrinolysin produced by strain 693 in 2% glucose-broth is inhibited by undiluted spinal fluid, undiluted normal serum and streptococcal antiserum; the potency of the antiserum does not exceed that of normal serum. In contrast, the streptococcal antiserum has a very marked neutralizing effect upon the fibrinolysin produced in 0.05% glucose-broth, although the influence of normal spinal fluid and normal serum upon this type of fibrinolysin is almost negligible.

The fibrinolysins produced in 2% glucose-broth by *S. viridans, enterococcus* and *pneumococcus* inhibited constantly the clotting of both human and animal plasma, but only when undiluted culture

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[7] Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 239.

[8] Van Deventer, J. K., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1117.

†Scarlet Fever Streptococcal Antitoxin, Lederle.

TABLE II.

Inhibitory Effect of Spinal Fluid, Normal Serum and Streptococcus Antiserum on Hemolytic Streptococcal Fibrinolysins.

Decreasing amounts of spinal fluid or of serum	Strain 693 in 2% glucose-broth						Strain 2327 in 0.05% glucose-broth					
	A Normal human spinal fluid		B Normal horse serum		C Streptococcal antiserum		A Normal human spinal fluid		B Normal horse serum		C Streptococcal antiserum	
	a	b	a	b	a	b	a	b	a	b	a	b
1. 0.5	—	—	—	—	—	—	—	++++	—	++	—	—
2. 0.1	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	—
3. 0.02	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	—
4. 0.004	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	+
5. 0.0008	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	+++
6. 0.	++++	++++	++++	++++	++++	++++	—	++++	—	++++	—	++++

Read after a—10 minutes 37° C.
b— 5 hours ”

+ - ++++ = various degrees of fibrinolysis.
— = no fibrinolysis (coagulation).

was used. Attempts to neutralize these fibrinolysins by means of specific antisera (especially with type-specific pneumococcal serum and its corresponding strain) thus far were not successful. The fibrinolysins of these microörganisms have the same properties as the fibrinolysin which is produced by hemolytic streptococci in 2% glucose-broth only.

Conclusions. 1. Hemolytic streptococci of human origin, when tested for fibrinolytic activity in 2% and 0.05% glucose-broths, may be classified in one of the following groups: (a) production in 2% only; (b) in 0.05% only; (c) in both 2% and 0.05%; and (d) no production in either.

Properties of the 2% glucose-broth fibrinolysin (a): This type of fibrinolysin inhibits clotting; it is effective toward human and animal plasma, but only when used undiluted; it is thermostable; and was not neutralized by specific streptococcal antisera.

The fibrinolysin of groups (b) and (c), even in high dilutions, lyses human plasma but not animal plasma; it is thermostable, and may be neutralized by streptococcal antisera.

Serum-broth fibrinolysin resembles 0.05% glucose-broth fibrinolysin, but is heat-labile.

2. *S. viridans, enterococcus,* and *pneumococcus,* when cultivated in 2% glucose-broth, may produce a fibrinolysin which has the same properties as the fibrinolysin produced by hemolytic streptococci in 2% glucose-broth.

TABLE III.

Properties of Different Fibrinolysins Produced by Streptococci of Human Origin in

I 2% glucose-broth	II 0.05% glucose-broth	III Serum-broth
Not specific for *Streptococcus hemolyticus*	Specific for *Streptococcus hemolyticus*	Specific for *Streptococcus hemolyticus*
Inhibition of plasma coagulation	Fibrinolysis	Fibrinolysis
Low titer	High titer	High titer
Lysis of human and animal plasma	Lysis of human plasma only	Lysis of human plasma only
Heat-resistant	Heat-resistant	Heat-labile
Not neutralized by streptococcal antiserum	Neutralized by streptococcal antiserum	Neutralized by streptococcal antiserum

8821 P

Experimental Hypertrophy Without and With Digitalization on Creatine Content of Rabbit Hearts.*

George M. Decherd, Jr., George Herrmann and E. H. Schwab.
From the Department of Medicine, University of Texas, Galveston.

In the type of cardiac hypertrophy resulting from nutritional anemia,[1] or following thyroid administration,[2] produced in rats, the percentage of creatine in the heart muscle has been found to drop; the total amount of creatine in the whole heart seemed to remain the same, the percentage decreasing as the heart muscle increased in weight. In a preliminary note,[3] we have recorded the creatine values in hearts in which hypertrophy has been produced by a different method. Using rabbits, the aortic leaflets were torn or punctured, thus producing experimental aortic regurgitation. The degree of hypertrophy has been determined by comparing the Heart-weight/Body-weight ratio with our previously established normal value.[4] The animals were of nearly the same age, and weighed around 3,000 gm. at the completion of the experiment. Creatine determinations were made following the technique of Seecof, Linegar and Myers.[5] In Table I summarized are the creatine values for the completed series of these animals. Some of them have been digitalized, in order to note the effect of this drug on hypertrophy; the remainder have had no digitalis. A control group was injected with digitalis, without having been operated upon.

In animals weighing from 2,500 to 3,500 gm., the normal hearts contain 176 mg.% creatine, with a standard deviation of 15.8 mg.%. Solids average 22.1%, S. D. 1.4%. The creatine calculated on the basis of dried weight averaged 825 mg.% ±80 mg.%. In the hypertrophied hearts, the average value is slightly higher, with a tendency for the higher values to be found in hearts with moderate hypertrophy, dropping to normal or slightly below as the grade of hyper-

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* Aided by Grant 349 of the American Medical Association.

[1] Cowan, D. W., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 417.

[2] Cowan, D. W., *Am. J. Physiol.*, 1934, **109**, 312.

[3] Decherd, G., Schwab, E. H., Herrmann, G., and Brown, W. O., Proc. Soc. Exp. Biol. and Med., 1936, **33**, 521; Herrmann, George, Decherd G., Schwab, E. H., and Erhard, P., Proc. Soc. Exp. Biol. and Med., 1934, **33**, 522.

[4] Herrmann, G., Decherd, G., Erhard, P., and Schwab, E. H., Proc. Soc. Exp. Biol. and Med., 1935, **33**, 409.

[5] Seecof, D. P., Linegar, C. R., and Myers, V. C., *Arch. Int. Med.*, 1934, **53**, 574.

TABLE I.

Creatine Content of Rabbit Hearts in Experimental Hypertrophy.

No. of Animals	Exper. Condition	Left Ventricle			Right Ventricle		
		mg. % Creatine	% Solids	mg. % Dried Value	mg. % Creatine	% Solids	mg. % Dried Value
14	Controls	176 ± 15.8	22.1 ± 1.4	825 ± 80	170	22	800
16	Digitalis 0.1 C.U.	203 ± 20.	22.4 ± 0.62	908 ± 58	196 ± 22	23.6 ± .64	831 ± 83
29	,, 0.3 or 0.5 C.U.	178 ± 15.	21.5 ± 0.79	824 ± 69			
13	Exp. hypertrophy	179 ± 18.	21.7 ± 0.37	828 ± 62	179 ± 26	22.3 ± .54	803 ± 117
14	Early hypertrophy + digitalis	218 ± 29.	22.6 ± 0.78	962 ± 125	208 ± 26	23.0 ± .92	904 ± 91
29	Hypertrophy of varying degrees + digitalis	186	23.0	839	185	22.7	822

trophy increased. This means that the absolute amount of creatine in such hypertrophied hearts is definitely increased.

In those digitalis control animals who received 0.1 cat unit twice weekly, injected as Digalen or Digifoline, there was a distinct increase in the cardiac creatine content. Those that received larger doses, namely 0.3 or 0.5 cat unit, showed no relative elevation.

In one group of digitalized hearts there was found a definite but small lesion, the grade of cardiac hypertrophy being doubtful, however, when judged by the ratio of heart weight to body weight. These hearts show markedly elevated creatine values. In the digitalized hearts with distinct hypertrophy, the degree depending on the size of the experimental lesion, there is on the average slightly higher creatine percentage than normal, demonstrating again the fact that there is an absolute increase in the amount of creatine in these hypertrophied hearts.

8822 C

Studies in Histochemistry. VIII. Relationship Between Concentration of Vitamin C and Development of Pineal Gland.

DAVID GLICK AND GERSON R. BISKIND.

From the Pathological and Research Laboratories of the Mount Zion Hospital, San Francisco.

Although there have been many investigations of the occurrence of vitamin C in animal tissues in recent years, there have been no studies, as far the authors have been able to ascertain, of the vitamin C in the pineal gland. This probably is due to the fact that the gland is very small and usually difficult to obtain. In the fully developed cow it weighs but a few hundred mg.

It should be of interest to compare the vitamin C content of the pineal with that of other nervous tissues or organs of internal secretion such as the adrenal medulla, pars nervosa of the hypophysis, and brain, all of which are somewhat related embryologically. It should also be desirable to determine the concentration of vitamin C in the pineal gland in relation to its various stages of development from fetus to adult. The presentation of these determinations and comparisons is the purpose of the present investigation.

Using the Linderstrøm-Lang, Holter microtitration apparatus,[1,2] and the micromethod for the estimation of vitamin C with 2,6-dichlorophenol indophenol[3], it was found that many determinations might be made on each gland with sufficient material left over for histological study as well.

The advantage of employing bovine tissue for this investigation depends (as in the case of the other organs studied) upon histological similarity to the human gland, availability in the fresh state, and, particularly in this case, convenient size As usual the glands were stored at —5°, and used for titration within one day.

When possible, a cylinder of tissue was cut out of the widest part of the pineal with a cork borer having a diameter of 4.2 mm., and the tissue was mounted on a freezing microtone, sections 30 μ thick were made, and every 5th section taken for titration. Because of the thinness of the gland, usually only 5 titration sections could be obtained in this manner, but the tissue was so homogenous that the titration values of the sections from any one pineal did not vary more than 0.20 c.mm. The accuracy fell with the lower titration values, but the low concentration of the vitamin in this gland makes lowered accuracy inevitable.

However, the pineals from fetuses, calves, and some of the cows were too small to permit use of the cork borer for sampling. In these cases the whole gland was frozen on the head of the microtone, and tissue was trimmed away with a scalpel so as to leave a block of tissue with a rectangular cross section on the microtome. The dimensions of this rectangle were determined with a vernier caliper so that the volume of each 30 μ section might be obtained. The smallest sections made in this manner were 4.5 x 3.1 mm., and the largest 8.0 x 4.8 mm. The tissue trimmings were saved for histological examination, as were the portions of tissue from the larger pineals left after removing the samples with the cork borer.

The material saved for histological examination was fixed in Bouin's fluid, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and mounted.

The histological changes that occur in the development of the bovine pineal gland as demonstrated by the hematoxylin-eosin stain are briefly summarized: In the 40 cm. fetus (5.2 months old) the pineal is composed of small clusters or rosette-like arrangements of cells that have a uniform appearance. They are separated by a

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[1] Linderstrøm-Lang, K., and Holter, H., in Nord, F. F., and Weidenhagen, R., *Ergebrisse der Enzymforschung*, Leipsig, 1934, **3**, 309.

[2] Glick, D., *J. Chem. Educ.*, 1935, **12**, 253.

[3] Glick, D., *J. Biol. Chem.*, 1935, **109**, 433.

TABLE I.

The Vitamin C Concentration in the Bovine Pineal at Different Stages of Development.

No. Animal	L 40 cm. Fetus (5.2 mo.)	C 70 cm. Fetus (7.2 mo.)	I 80 cm. Fetus (8 mo.)	D Calf	E Calf	M Calf	N Calf	O Calf	A Cow	B Cow	K Cow
Aver. titration per section. c.mm. dye minus .60 c.mm. blank	.50	.80	.90	.53	.40	.45	.54	.52	.40	.37	.22
Vitamin C per section, micrograms	.06	.10	.11	.07	.05	.06	.07	.07	.05	.05	.03
Mg. vitamin C per gm. tissue	.15	.24	.27	.16	.12	.15	.16	.16	.12	.12	.07

scant fibrillar substance. In the 80 cm. fetus (8 months old) the cells have a more distinct rosette arrangement, but they are slightly larger; cells are often situated around the capillaries. In the calf the several types of cells are uniformly distributed between irregular bundles of fibrillar material and the rosette formations have disappeared. In the adult this variety of cell types is more clearly distinguished, and glial fibrils are present.

From Table I it may be seen that the vitamin C concentration increased from 0.15 mg. per gm. of tissue in the 5.2-months fetus to a maximum of 0.27 mg. per gm. in the 8-months fetus. The concentration then decreases through the calf to the adult stage, reaching its lowest level of 0.07-0.12 mg. per gm. in the older animals. Thus the general principle that the vitamin C concentration in organs falls with increasing age as demonstrated by King and coworkers,[4] von Euler and Klussmann,[5] and Plaut and Bülow,[6] applies to the pineal gland as well. The apparent exception to this rule in the case of the thymus, if only the glandular tissue is considered, has been pointed out in a preceding paper.[7]

If a comparison is made of the vitamin C concentrations of the brain[6,8] and pineal gland, it is found that they are approximately the same. These values however are much lower than those observed for the adrenal medulla[9] or pars nervosa of the hypophysis.[10] Hence there appears to be no relationship between the vitamin C concentration in nervous tissues in different parts of the body.

It is not known whether vitamin C is involved in the production or function of the hormone found in the pineal gland. But it may be considered probable that the vitamin is concerned with the respiration of the pineal tissue just as it seems to be concerned with the respiration of brain tissue.[8,11]

Summary. The concentration of vitamin C has been determined in the bovine pineal gland at various stages of development from the early fetus (just over 5 months old) to the adult. Histological examinations have been made at these various stages in an attempt to correlate the cellular state with the concentration of vitamin C present.

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[4] Yavorsky, M., Almaden, P., and King, C. G., *J. Biol. Chem.*, 1934, **106**, 525.

[5] von Euler, H., and Klussmann, E., *Z. physiol. Chem.*, 1933, **217**, 167; 1933, **219**, 215.

[6] Plaut, F., and Bülow, M., *Z. Neurol.*, 1935, **152**, 84.

[7] Glick, D., and Biskind, G. R., *J. Biol. Chem.*, 1936, **114**, 1.

[8] Plaut, F., and Stern, K., *Naturwissenschaften*, 1935, **23**, 557.

[9] Glick, D., and Biskind, G. R., *J. Biol. Chem.*, 1935, **110**, 1.

[10] Glick, D., and Biskind, G. R., *J. Biol. Chem.*, 1935, **110**, 583.

[11] Malmberg, M., and von Euler, H., *Z. physiol. Chem.*, 1935, **235**, 97.

The authors wish to express their appreciation to Mr. J. L. Artigues, of the Bayle-Lacoste Co., San Francisco, for furnishing the glands used in this investigation.

8823 C

Effect of Administration of Desiccated Thyroid During Pregnancy in the Albino Rat.

D. N. DANFORTH AND S. LOUMOS. (Introduced by A. C. Ivy.)

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago.

Several years ago the observation was made by one of us (S.L.) that pregnant rats which received desiccated thyroid did not show the rather marked reduction in weight noted in similarly treated animals which were not pregnant. This observation has been extended and the rate of oxygen consumption under comparable conditions has been determined.

Methods. Thirty-three young, mature rats of approximately the same weight (180 gm.) and age and from the same colony were used. These were divided into 3 groups of 10, 11, and 12 rats respectively. After a period of 5 days of control feeding of the same amount (15 gm. daily) of a standard diet (Steenbock), they were assumed to be in a normal, pre-experimental condition. All rats received the same diet and the amount given above. The only variables in the three tests were pregnancy and the feeding of desiccated thyroid.

The *first group* were *not mated* and received in their diet daily 100 mg. of *desiccated thyroid.* This was continued for a period of 16 days, during which time body weight and rate of O_2 consumption were determined at 2- to 3-day intervals. This group served as *thyroid-fed, non-pregnant controls,* a 16-day period being chosen because that was the period of treatment and observation in the thyroid-fed, pregnant animals.

The *second group were mated* and given the same diet *without thyroid.* Determinations of O_2 consumption and body-weight were started after mating and continued to delivery. This group served as *non-thyroid fed, pregnant controls.*

The *third group were mated* and given the same diet *with thyroid.* The thyroid was not given until from 5 to 10 days after placing the female with the male, or until we were certain that the rats were pregnant. It is for this reason that the 16-day period of thyroid feeding is shown in the figure.

The apparatus used for measuring oxygen consumption was a closed system[1] consisting of a bell jar with enclosed soda-lime tray, the former resting on a wooden base. The base was perforated by a copper tube connected to a spirometer from which readings of cc. of O_2 used could be measured directly. The rats were fed about 4 p.m. and the O_2 consumption determined about 12 a.m. This was done because we were interested only in comparative rates of O_2 consumption and believed that more comparable rates could be obtained in "hyperthyroid" rats by not fasting them for 16 or 24 hrs., as is usually done in determining basal metabolism. The rate of O_2 consumption in the graph (Fig. 1) is expressed in cc. of O_2 per 200 gm. of body-weight per hr. for purposes of comparison, the data being shown to be statistically significant.

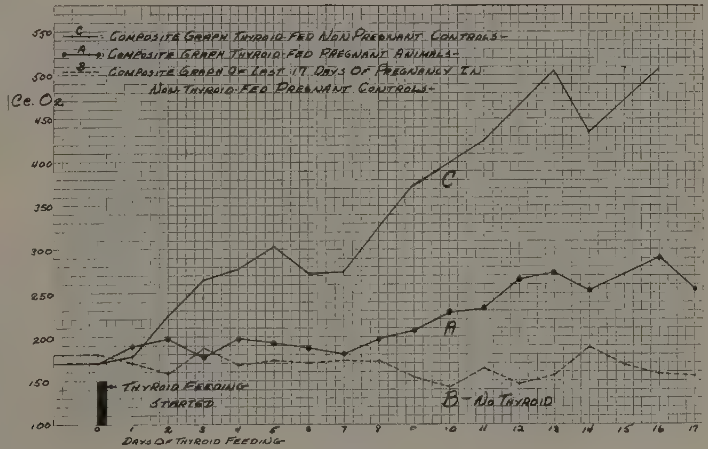

Fig. 1.

Results. Loss of weight. Of course, the non-pregnant rats fed thyroid (Group I) lost weight. During a 12-day period of thyroid feeding, they lost 24 gm. (av.) per 200 gm. of body-weight. However, the pregnant rats fed thyroid (Group III) during a comparable 12-day period did not lose weight, but gained 47 gm. (av.) per 200 gm. of body weight. During the entire period of pregnancy the non-thyroid fed, pregnant controls (Group II) gained 61 gm. (av.).

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[1] Tainter and Rytand, Proc. Soc. Exp. Biol. and Med., 1934, **32**, 361.

Thus, pregnancy prevents loss of weight in the rat receiving 100 mg. of desiccated thyroid per day; in fact, the pregnant rats receiving this dose of thyroid actually gained about as much weight as the pregnant controls for an analogous period of time. (The 15 gm. daily ration was entirely consumed by the thyroid-fed rats, but not always by the non-thyroid-fed.)

Oxygen consumption. The averaged results of the O_2 consumption rates are shown in the accompanying figure. By the method we employed, pregnancy had little effect on the rate of O_2 consumption (Curve B, graph). By comparing curves A and C, it is clear that the rate of O_2 consumption in the thyroid-fed, non-pregnant control rats was definitely greater than that of the thyroid-fed, pregnant rats, both of which groups received the same amount of food and thyroid daily. On comparing Curve A with Curve B it is evident that the thyroid fed to the pregnant rats (Curve A) increased somewhat the rate of O_2 consumption. In all probability had the dose of thyroid been smaller, Curve A would have approximated Curve B. Thus, by determining that amount of thyroid fed to a pregnant rat which would not cause an elevation in O_2 consumption, one might approximate or obtain an index of the extra-demand for thyroid secretion created by the state of pregnancy.

Summary and conclusions. Three groups of mature female rats of approximately the same age and weight and from the same colony were placed on the same amount of a standard diet. One group served as thyroid-fed (100 mg. desiccated thyroid), non-pregnant controls; the second group served as non-thyroid fed, pregnant controls; the third group, for about 16 days prior to parturition, received 100 mg. desiccated thyroid daily. The first group lost 24 gm. (av.) of weight in 12 days and the O_2 consumption markedly increased; the second group gained 61 gm. of weight during the entire period of pregnancy and the O_2 consumption did not change appreciably; the third group gained 47 gm. during the comparable period of 12 days used in the first group, and the O_2 consumption was increased, but not to the extent as in the first group. Thus, according to the criteria of body weight and O_2 consumption pregnant rats are more tolerant to desiccated thyroid than non-pregnant rats. The mechanism of the increased tolerance remains for the present unknown.

8824 C

Changes Occurring in the Blood Picture During Fetal Life.

JULIA MARY JONES, MARY E. SHIPP AND THOMAS A. GONDER, JR.
(Introduced by F. H. Swett.)

From the Department of Anatomy, Duke University School of Medicine.

While studying the blood of newly born infants, the characteristics of the blood picture at that period were observed to differ from those of the adult in a very interesting manner. In brief, the erythrocytes were present in greater numbers, were of greater mean volume, and the hemoglobin content was higher than normally seen in human adults. These observations raised the question concerning the nature of the factors which effect those alterations between the blood pictures of the 2 periods of life. The possibility was also suggested that a clear understanding of these factors might aid in the elucidation of the mechanisms of various blood dyscrasias of adult life. With these ideas in mind it seemed advisable to establish first the normal course of blood changes which occur in the fetus during intra-uterine life, and then later to investigate the mechanism of their production. The present work deals with a series of blood studies on embryos of the domestic pig, *Sus scrofa.*

This experimental animal was chosen because its blood is very similar to that of the human and because experimentation of recent years has demonstrated the presence of the so-called anti-anemic principle in the mucosa of the hog stomach. This latter fact suggested the possibility that this substance may exert a similar controlling effect on the hematopoietic function in the pig and in the human—as well as in various other animals less adaptable to laboratory experimentation. Support has been given to this idea by the work of Miller and Rhoads[1] who, working with the pig, produced a form of hyperchromic anemia with associated gastric anacidity by means of dietary restriction. Extract made from the livers of these anemic animals was not therapeutically effective when administered to patients suffering from pernicious anemia.

While work on the present series was in progress, Wintrobe and Shumacker[2] published a large series of blood studies on fetuses of various animal species, including the pig. Our findings corroborate their observations on the latter, and since a thorough review of the pertinent literature was included in their paper it will be omitted here.

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[1] Miller, D. K., and Rhoads, C. P., *J. Clin. Invest.*, 1935, **14**, 153.

[2] Wintrobe, M. M., and Shumacker, H. B., Jr., *Am. J. Anat.*, 1936, **58**, 313.

Pig embryos were obtained from the abbatoir immediately after they had been removed from the parent animal, and the hearts were still beating when blood specimens were drawn by cardiac puncture. The study included three embryos from each of eighteen litters which ranged in age from 42 to 106 days of gestation as estimated from the crown-rump length.[3] Variations in the size of litter mates were frequent and some variations in the blood picture were noted among animals of the same approximate age. Immediate studies by two observers included red blood counts, hemoglobin determinations and reticulocyte counts. The hemoglobin determinations were made by the acid-hematin method, using Hellige hemometers corrected by Van Slyke oxygen saturation so that 15.5 gm. of hemoglobin per 100 cc. blood were equivalent to 100%. The reticulocyte counts were made on fresh blood smears stained by brilliant cresyl blue. Fixed Wilson-stained smears were prepared for study

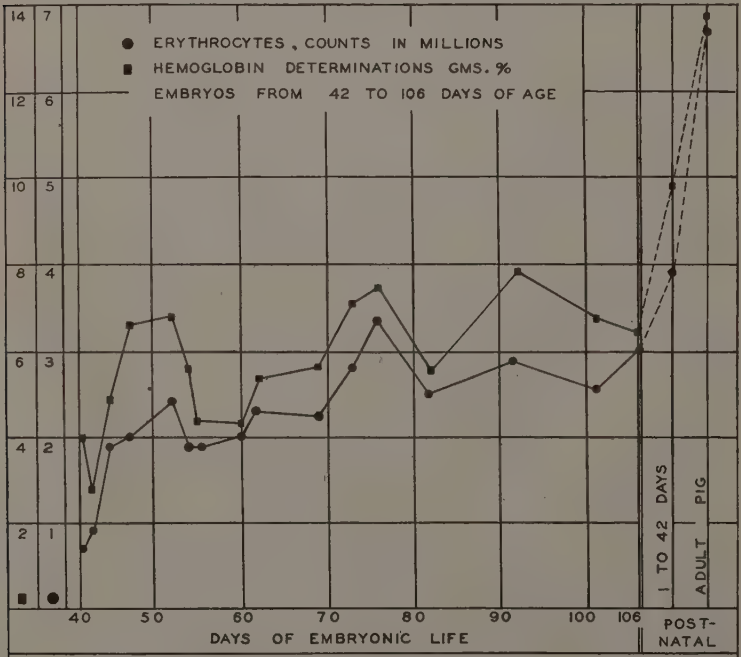

FIG. 1.

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[3] Warlick, B. L., *J. Morph. and Physiol.*, 1928, **46**, 59.

and for cell measurements. Hematocrit determinations[4] were made on oxalated blood and mean corpuscular volumes and mean corpuscular hemoglobins were calculated from the results.

In the earliest embryos studied (42 days gestation) the erythrocyte counts and hemoglobin determinations were low (Fig. 1). At this time the mean corpuscular volume and mean corpuscular hemoglobin were high (Fig. 2). Characteristic erythrocytes of this period were large (Fig. 3) and showed striking variations in size

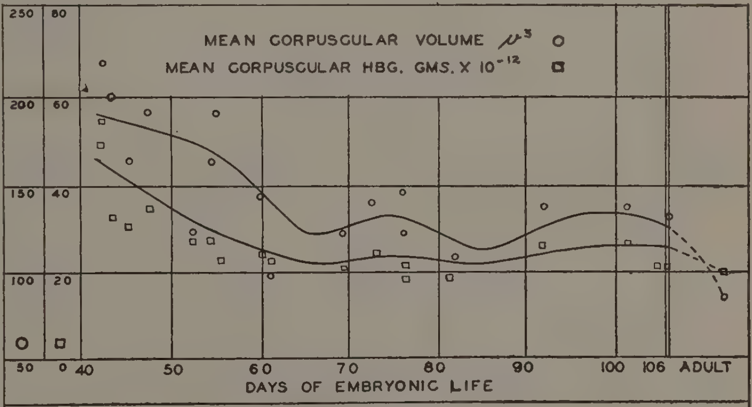

FIG. 2.

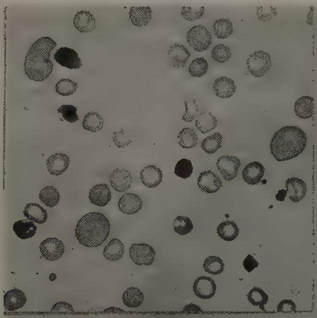

FIG. 3.

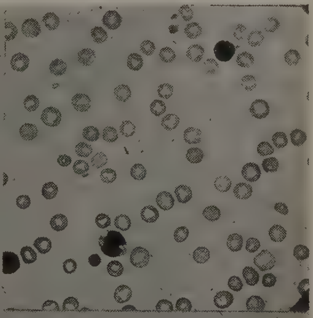

FIG. 4.

Fig. 3.

Macrocytosis with marked variations in size and shape of erythrocytes at 42 days. Wilson Stain (Photomicrograph x 375).

Fig. 4.

Blood at 106 days showing relative and actual decrease in size of erythrocytes with greater uniformity of contour. Wilson Stain (Photomicrograph x 375).

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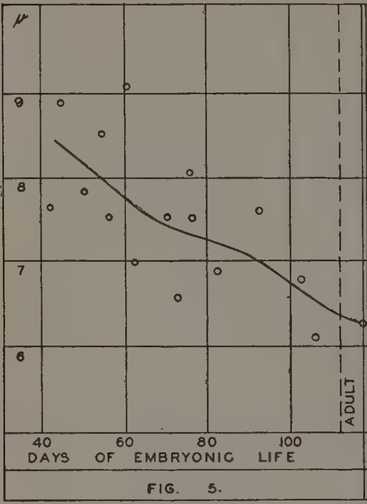
[4] Wintrobe, M. M., *Am. J. Med. Sci.*, 1929, **177**, 513.



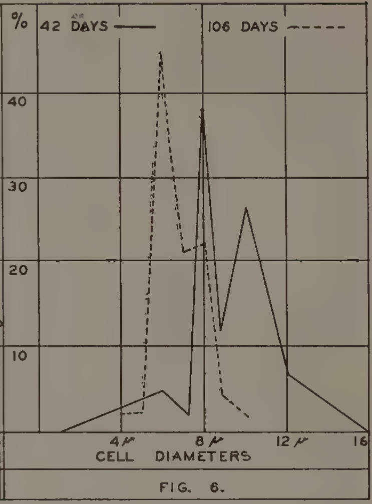


FIG. 5.
Average diameter, in micra, of erythrocytes at various stages of embryonic life.

FIG. 6.
Distribution of erythrocyte diameters at 42 and 106 days of embryonic life.

and form. Many bizarre immature forms were seen and many of these were deeply basophilic. Reticulocytes were numerous in the blood of the very young embryos.

As the period of gestation lengthened, interesting changes were noted. There was a steady increase in the total number of erythrocytes and in the amount of hemoglobin. At the same time there was a fall in mean corpuscular volume; and the mean corpuscular hemoglobin, which had been high in the youngest embryos, fell rapidly so that after the 60th day it never rose above 25×10^{-12} gm. The erythrocytes became progressively more regular in size and shape (Fig. 4), while fewer and fewer reticulocytes, nucleated cells and basophilic cells were seen in the circulating blood. In addition to a decrease in the average cell diameter (Fig. 5) as the period of gestation increased, the erythrocytes became more uniform in size (Fig. 6). Table I further demonstrates the changes seen between the bloods of the youngest and oldest embryos of the series. For sake of contrast, similar figures obtained by other workers for newly born and adult pigs have been included.

These findings are essentially in agreement with those of Wintrobe and Shumacker.[2] In another study Wintrobe and Shumacker[6] pointed out that the changes which occur in the blood dur-

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[6] Wintrobe, M. M., and Shumacker, H. B., Jr., *J. Clin. Invest.*, 1935, **14**, 837.

TABLE I.

Age	42 days	106 days	2-42 days post-natal	adult*
Total erythrocyte count in millions	0.74	3.0	3.9	6.8
Hemoglobin in grams %	3.6	6.76	9.7	14.0
Mean corpuscular vol. in cubic micra	216.0	128.0	—	72.0
Mean corpuscular hemoglobin in gm. x 10^{-12}	56.2	21.0	—	20.0
Average cell diam. in micra	8.9	6.01	—	6.3
Range of cell diam. in micra	2.0-16.0	4.0-8.5	—	—
Reticulocytes % (1000 cells counted)	28.8	8.0	—	—

*These figures have been compiled and calculated from several sources.[5, 1]

ing fetal life suggest very strikingly the changes which result in the blood of pernicious anemia patients when they are subjected to liver therapy. Certainly the blood picture of the early pig embryos included in the present series is so similar to that seen in such patients as to make a distinction difficult if a comparative study were undertaken. The presence of hyperchromic, macrocytic anemia; with high mean corpuscular volume and hemoglobin, associated with the appearance of immature erythrocytes in the circulating blood stream; would seem to satisfy the hematological criteria for making a diagnosis of pernicious anemia. The changes which follow in the blood of the fetus are indeed very similar to those which result from liver therapy in pernicious anemia. The exact relationship of the two states is only a matter of conjecture at present, but there seems to be justification for suggesting that the hematopoietic tissues of the fetus are influenced by the same anti-anemic factor which, when administered therapeutically to the patient, results in the familiar alterations in the blood picture of pernicious anemia. Perhaps the factor is absorbed from the mother or it may be elaborated by the fetus. Additional information is necessary before any conclusions on this point can be reached. Perhaps some evidence might be obtained by a study of fetal blood formation in the absence of an adequate maternal supply of the anti-anemic factor.

We wish to express our appreciation to Dr. Lewis H. Weed of the Department of Anatomy at the Johns Hopkins Medical School for making possible the collection of the material upon which this work is based.

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[5] Palmer, C. C., *J. Agr. Res.*, 1917-1918, **9**, 131.

8825 P

Growth in Adrenalectomized Puppies on Diet Low in Potassium, High in Sodium Chloride, Sodium Citrate.

W. D. ALLERS AND L. A. CRANDALL, JR.

From the Department of Physiology and Pharmacology, Northwestern University Medical School, Chicago, Illinois.

That the adrenal cortex or the cortical hormone is not necessary for maintenance of adrenalectomized adult dogs, adequately supplied with sodium chloride and sodium bicarbonate (or better, sodium citrate) was reported in June, 1935, by Harrop *et al.*,[1] and by one of us.[2] While Harrop and his coworkers paid little attention to the concentration of potassium in the diet, Allers[2,3] fed synthetic diets low in potassium and was able to sustain more normal balances in electrolytes provided sufficient sodium salts were added to the diet. That feeding small amounts of a potassium salt suddenly produces typical symptoms of insufficiency in such treated animals has very recently been shown by Allers, Nilson and Kendall.[4] The latter investigators have further shown that the ability to reproduce is also normal in both the adrenalectomized male and female dogs maintained in good condition by sodium salts and a low potassium diet.

We now report that growth also occurs in adrenalectomized puppies in the absence of cortin. Grollman and Firor[5] have stated that growth ceases after adrenalectomy in rats, cats and dogs that are maintained in a state of "chronic insufficiency" by giving only small amounts of cortical hormone. This observation is not invalidated by our results, since our animals have not presented indications of insufficiency, except occasionally when sodium salts were withheld for short periods. Our data show that following the removal of the second adrenal and without the administration of any cortical hormone after the first or second day, 2 puppies, when fed a diet high in sodium chloride and sodium citrate but low in potassium steadily gained in body weight. The diet consisted of 54.5% cracker meal, 17% crude casein, 20% lard, 6% bone ash, and

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[1] Harrop, G. A., Soffer, L. J., Nicholson, W. M., and Strauss, M., *J. Exp. Med.*, 1935, **61**, 839.

[2] Allers, W. D., *Proc. Staff Mayo Clin.*, 1935, **10**, 406.

[3] Allers, W. D. (in press).

[4] Allers, W. D., Nilson, H. W., and Kendall, E., *Proc. Staff Mayo Clin.*, 1936, **11**, 283.

[5] Grollman, Arthur, and Firor, W. M., *Am. J. Physiol.*, 1935, **112**, 310.

2.5% pasteurized brewer's yeast. Each day the puppies were fed 90 calories per kilo body weight of the ration, to which were added 10.7 mg. MgO, 1.5 mg. NaI, and 15 mg. ferric citrate, per kilo body weight, and 15 cc. of cod liver oil. Fresh liver was occasionally added to the diet, but was withheld when it provoked symptoms of weakness. The first animal has reached almost mature size (3 months after the second operation) and has remained in excellent condition except when the sodium salts were experimentally reduced, at which times weakness and anorexia promptly ensued. Its body weight increased from 20 to 30 lbs. The second puppy, operated at an earlier age (about 3 months) has also grown rapidly, gaining 65% of its original body weight within 48 days, and was sensitive to a decreased intake of sodium salts. X-ray records demonstrate growth in the skeletal bones, and teeth.

To maintain adrenalectomized adult dogs without cortin requires the exercise of meticulous care, as emphasized elsewhere,[3] but the task is even greater when puppies are used, since their stomachs are extremely sensitive to the high concentration of salts in the diets. To avoid nausea the salts were incorporated in a medium that remained quite impervious to gastric juice but slowly dissolved in the intestine. One mixture found to be satisfactory was powdered sodium chloride, powdered sodium citrate, and powdered commercial gastric mucin, which, after intimate mixing, were carefully mixed with stearic acid heated several degrees above its melting point. After solidification the mass was broken into particles approximating the size of a kidney bean. Weighed portions of this salt mixture were added to the daily diet low in potassium. Whether this mixture prevents nausea depends upon the ratio of the constituents. Usually the following proportion was satisfactory: 1 part mucin, ½ part NaCl, ½ part Na-citrate, and 1 part stearic acid. The puppies required 20 to 30 gm. of such a mixture, in addition to 0.5 to 2 liters of 0.7% saline daily.

8826 C

Temporary Suppression of Estrous Cycles in the Rat by Prolactin.

Ernest L. Lahr and Oscar Riddle.

From Carnegie Institution, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Though prolactin was early shown to induce a speedy and extreme atrophy (decrease in weight) of the gonads of adult birds,[1,2] of both sexes, this hormone was found to have no influence whatever upon the weights of testes of adult rats.[3] Because of the relation of this hormone to lactation it has nevertheless seemed probable that the "lactation anestrus" of mammals is associated with this pituitary hormone—and most plausibly with its proved depressant action on the gonads of some animals (birds). The present study, following that of Dresel,[4] demonstrates that daily injections of prolactin temporarily suppress those ovarian functions which are expressed by estrus. In addition, it is here shown that a dosage of progesterone which (in another study) appears to have an influence on maternal behavior in the non-parous rat has no appreciable influence on the estrous cycles of these rats.

In mature non-parous mice Dresel briefly reported that "prolactin suspends the estrous cycle for about 3 weeks, after which a prolonged estrum sets in lasting from 4 to 8 days in spite of further continued injections." These observations led to the suggestion that the suspension of the estrous cycle during lactation is caused by prolactin, rather than by the corpus luteum hormone.

Smears were made by us once daily on mature non-parous rats, and the vaginal material was obtained by pipette. Observations were made on 3 series of prolactin-injected rats. First, a preliminary series of 4 rats 172 days old at first injection. Previously smeared for 16 days, the cycles of 3 of these 4 rats had been found quite regular and normal; thereafter they each received 4 units of prolactin daily for 15 days, and each rat showed partial or nearly complete suppression of 1 or 2 cycles followed by resumption of quite normal cycles. In the partial or "mixed" smears which some-

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[1] Riddle, O., and Bates, R. W., *Endocrin.*, 1933, **17**, 689.

[2] Bates, R. W., Lahr, E. L., and Riddle, O., *Am. J. Physiol.*, 1935, **111**, 361.

[3] Riddle, O., Lahr, E. L., Bates, R. W., and Moran, C. S., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 509.

[4] Dresel, I., *Science*, 1935, **82**, 173.

times replaced the normal cycles there were few or no squamous cells. The cycles of two control rats of this series were normal and unmodified during this period. Second, a series of 9 rats whose response is shown in Fig. 1. The cycles of the 4 control rats (not figured) of this series remained nearly or quite regular and normal. Of the 9 treated rats, aged 148-186 days, 3 (here called A-C) were injected with 30 units of prolactin daily for 35 days (19 days for 1). About 3 cycles were suppressed in each rat, but despite continued injections quite normal cycles recurred between the 13th and 16th day of treatment; in the two rats treated for 35 days the 3 next following cycles also were completely suppressed, after which quite normal cycles occurred although injections were being continued. Rats D-I received 15 units of prolactin daily; in 3 of the 4 rats treated for 28 days, about 4 cycles were completely suppressed (in the other rat only 2 or 3 cycles), and thereafter a single normal cycle was followed by a further suppression of cycles in all 4 cases. Two rats (H, I) received only 8 and 12 such injections, and were killed at these periods for a special study of their ovaries which, in both cases, disclosed the retention of large corpora with large cells apparently not in stages of degeneration. A third series is formed from random observations on a number of rats injected with pro-

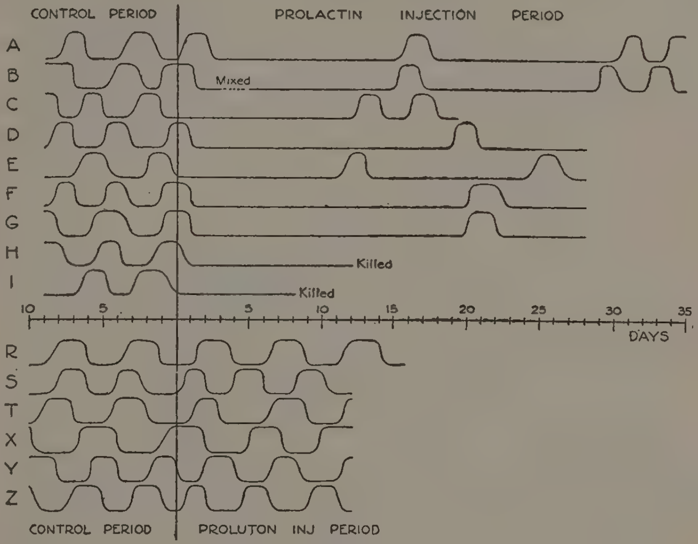

Fig. 1.

lactin for shorter periods and with variable dosage. On these rats the infrequency of cycles under such treatment has been confirmed.

It can be observed that prolactin dosage was initiated at all of the various stages of the cycle in one or another of the 9 rats recorded on Fig. 1. All prolactin preparations used on those 9 rats, and on the 4 rats of the preliminary series, had been heated to 60° C. for 5 hours after previously testing free from follicle-stimulating and thyrotropic hormones (dove testis and dove thyroid tests). Injections were made once daily and subcutaneously. It is particularly notable that after stopping the injections there was almost immediate resumption of normal estrus in all cases.

The 6 rats (Fig. 1) receiving progesterone* were 156-172 days old at the beginning of treatment. The dosage was 0.03 mg. daily, subcutaneously, for 12 to 16 days. The rhythm of the cycles was *not* interrupted, and the character of the smears remained normal. At a dosage level 10 times higher (0.3 mg. daily) we noted slightly prolonged diestrus (remained normal in 2 controls), and Selye, Browne and Collip[5] have already reported the suppression of estrus in the rat with huge doses (4 mg. daily) of progesterone.

It is of course possible that prolactin suppresses estrous cycles through diminishing the release of F.S.H. from the rat's own pituitary; but a direct and antagonistic action of these two hormones on the gonads of birds has not been excluded. Whatever the mechanism involved in its action in both mice and rats, prolactin has been found capable of temporarily suppressing ovarian cyclic activity; and, curiously enough, in both cases cycles are later resumed under continued injections. Whether increasing daily doses would prevent this resumption of cycles is still unknown. In the case of the rat the anestrous period induced by prolactin seems always to be rather shorter than the gestation or the lactation period. The retained large corpora found at 8 and 12 days of prolactin treatment suggest an active output of progesterone, but the failure of low or moderate amounts of this hormone to suppress cycles is here definitely indicated.

Conclusions. In the regulation of the ovarian activity of rats prolactin is shown to be capable of playing a part, directly or indirectly. Prolactin can not be summarily omitted from lists of pituitary principles having action on the gonads of mammals. The suggestion of Dresel that lactation anestrus in mice is induced by prolactin is supported by these tests on mature non-parous rats. But under effective prolactin dosage the corpora of rats remain large, and at the age of 10-12 days their large cells show no clear evidence of degeneration. The failure of daily injections of low or moderate doses of progesterone to affect the estrous cycles of rats suggests that in this rodent, though the corpus luteum hormone plays its part, it is perhaps not the hormone primarily responsible for the anestrous of either gestation or lactation.

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* Proluton, crystalline progesterone dissolved in oil, was supplied for these tests through the courtesy of Dr. Erwin Schwenk of the Schering Corporation, Bloomfield, N. J.

[5] Selye, H., Browne, J. S. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 472

8827 P

Removal of Corpora Allata on Egg Production in the Grasshopper, *Melanoplus Differentialis*.*

ISABELLE G. WEED. (Introduced by J. H. Bodine.)

From the Department of Zoology, State University of Iowa, Iowa City.

It has been shown by Wigglesworth[1] that in *Rhodnius prolixus* the corpora allata secrete a hormone which is necessary for the production of eggs. In order to determine whether these glands have a similar function in the grasshopper, females were operated as shown in Table I. For comparison, unoperated adults were killed at intervals from one to 42 days after moulting. Camera lucida drawings were made of the largest eggs in each of the above animals, egg length was measured, and the oviducts were examined to determine whether the secretion produced by the anterior portions of the oviducts was present. This secretion forms the ootheca.

There was great variation in the development of the ovary in unoperated adults autopsied from 28 to 42 days after moulting. However, of the 43 specimens examined, 35 contained eggs which were more than 1 mm. in length. Of these, the eggs of 8 were fully developed and had passed into the oviducts. The operated controls at autopsy also contained eggs exceeding 1 mm. in length and in one instance the fully developed eggs had entered the oviducts.

The eggs of the animals from which both corpora allata had been removed, however, were less than .9 mm. in length at autopsy in all

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* Aided by a grant from the Rockefeller Foundation for work on cellular biology.

[1] Wigglesworth, V. B., *Nature*, 1935, **136**, 338.

cases except those which had been operated at 16 and 23 days, suggesting that development of the eggs was suppressed by lack of the corpora allata. The slightly larger size of the eggs in those animals operated at 16 and 23 days was probably because they had reached a higher stage of development before operation, since a number of unoperated females killed between 16 and 23 days were found to contain eggs exceeding .9 mm. in length.

TABLE I.

No. Unoperated Animals Observed	Operated Animals No. of Glands Removed	Days after Moulting		Length of eggs in mm.	Secretion in Oviducts
		Operated	Autopsied		
	2	*	34	.79	Absent
	2	4	35	.61	,,
	2	4	40	.68	,,
	2	4	42	.77	,,
	2	4	42	.50	,,
	2	5	33	.83	,,
	2	5	42	.50	,,
	2	6	35	.48	,,
	2	6	42	.52	,,
	2	6	46	.86	,,
	2	7	28	.59	,,
	2	11	35	.82	Present
	2	13	35	.65	Absent
	2	14	42	.86	Present
	2	16	42	1.00	Absent
	2	23	42	1.08	Present
	1	6	28	.81	,,
	0	4	28	2.82	,,
	0	6	28	1.84	,,
	0	7	35	1.28	,,
	0	12	42	4.33†	,,
8			28-42	4-5.5†	,,
5			,,	4-5	,,
6			,,	3-4	,,
8			,,	2-3	,,
8			,,	1-2	,,
8			,,	0-1	,,

*Operated 4 days after entering the final nymphal stadium.
†Eggs fully developed and in the oviducts.

At autopsy, secretion was lacking in the oviducts of all the animals operated up to 11 days after moulting. This indicates that formation of the secretion is also inhibited by removal of the corpora allata, since both the operated and unoperated controls, as well as the animal from which only one gland had been removed, contained secretion in the oviducts at autopsy, even in those cases where the eggs had remained small. Secretion had probably already started in the oviducts of the animals operated at 11, 14, and 23 days and had not in those operated at 13 and 16 days. This is evidenced by the fact that secretion had begun in some of the unoperated animals examined at from 10 to 23 days, while in others it had not.

The results obtained in the above preliminary experiments, therefore, suggest that in *Melanoplus differentialis,* as in *Rhodnius prolixus,* the corpora allata are necessary to normal development of the eggs, and that, further, secretion in the glandular portion of the oviducts is also influenced by the corpora allata.

8828 P

Experimental Study of Moulting in the Grasshopper, *Melanoplus Differentialis.**

ISABELLE G. WEED. (Introduced by J. H. Bodine.)

From the Department of Zoology, State University of Iowa, Iowa City.

Wigglesworth,[1,2] working on a blood sucking hemipteron, *Rhodnius prolixus,* found that if the head were taken off before a certain "critical period" in a nymphal stadium, the animal did not moult, but if the head were removed after this time, the animal moulted as usual. On the basis of these and other experiments he concluded that a hormone is secreted in the head, probably by the corpus allatum (a single structure in *Rhodnius*), which induces moulting.

In view of this work it seemed of interest to determine whether moulting is controlled by the corpora allata in the grasshopper, *Melanoplus differentialis.* Therefore, the corpora allata (paired structures in this animal) were removed from a number of nymphs from 1 to 9 days after they entered the 6th and final stadium.

Since most of the unoperated nymphs observed spent from 15 to 20 days in the 6th stadium, it might be expected that animals operated up to the 5th day of this period would fail to moult, yet as shown in Table I, all of the nymphs from which both glands had been removed, including 3 specimens which were operated at one day, moulted from 17 to 23 days from the beginning of the stadium. In addition to the above animals, 8 others were operated at from one to 5 days and 6 at 6 to 7 days, but in these cases there was some doubt whether both glands had been removed, although in most

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* Aided by a grant from the Rockefeller Foundation for work on cellular biology.

[1] Wigglesworth, V. B., *Quart. J. Micr. Sci.*, 1934, **77**, 191.

[2] Wigglesworth, V. B., *Nature*, 1935, **136**, 338.

TABLE I.

No. of Glands Removed	Days after Entering the 6th Stadium	
	Operated	Moulted
2	1	21
2	1	23
2	1	18
2	2	18
2	3	20
2	3	23
2	5	21
2	9	17

instances it was fairly certain that they had been. These nymphs also moulted, requiring from 18 to 29 days in the 6th stadium. Moulting was slightly retarded in all of the operated animals as compared with the unoperated, but this may be accounted for by the severity of the operation since 5 animals operated at 0 to 5 days without removing the glands spent from 24 to 28 days in the 6th stadium.

Preliminary experiments, therefore, indicate that in *Melanoplus differentialis* the corpora allata do not influence moulting.

8829 C

Effect of High Concentrations of Glucose on Fibroblasts, Leucocytes, and Epithelial Cells.

ALBERT H. EBELING. (Introduced by Lillian E. Baker.)

From the Laboratories of the Rockefeller Institute for Medical Research, New York City.

Hyperglycemia, according to available evidence, does not damage the tissues. Kirby, Estey, and Wiener[1] noted no real difference in the growth of lens epithelium with a dextrose concentration as high as 478 mg. %, and it was only when the concentration was 578 mg. % that they observed a slight inhibition of growth. Carrel[2] failed to find any interference with tissue growth in media containing 300 mg. %. Willmer[3] states that, other things being equal, the amount of cell migration was found to be directly dependent on the amount of glucose present in the medium up to a concentration of 1,000 mg. %. He found that above that limit glucose appeared to become slightly toxic. Yen-lang Huang[4] found that d-glucose in low concentrations furthers the growth of fibroblasts; high concentrations inhibit it. Since the normal blood sugar concentration in healthy fowls averages about 200 mg. %, it was thought of interest to study the effect, from the standpoint of sugar tolerance, of abnormal glucose concentrations on 3 different cell types.

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[1] Kirby, D. B., Estey, K., and Wiener, R. von E., *Tr. Am. Acad. Ophth.*, 1932, **37**, 196.

[2] Unpublished experiments referred to by H. O. Mosenthal in Hyperglycemia, Evaluation in the Treatment of Diabetes Melitus, *J. Am. Med. Assn.*, 1935, **105**, 484.

[3] Willmer, E. N., *Brit. J. Exp. Biol.*, 1927, **4**, 280.

The tissues used are fibroblasts from a 24-year-old strain, iris epithelium, and adult chicken blood leucocytes. The tissue fragments and the leucocytic films are cultivated in 25% serum by the usual flask technique. The respective total concentrations of glucose are: controls, 200 mg. %; experiments, 390, 650, 1,150. 2,090, and 3,200 mg. %. All the media are isotonic, the concentration of the salts being reduced as that of glucose is increased.

Fibroblasts. After 2 days' incubation, cell migration is respectively 22, 17, and 20% greater in the glucose concentrations of 390, 650, and 1,150 mg. %, than in the controls. No apparent difference in the appearance of the cells is observed. At the concentrations of 2,090 mg. %, the cell migration is about 16% less than in the control, and the cells appear granular. In the 3,200 mg. % concentration, the migration is about 30% less than in the control, and the cells appear more granular. After 7 days' incubation, there is practically no difference in the extent of cell migration in the 390 and 650 mg. % concentrations as compared with the controls. In the 1,150 mg. % concentration, migration is about 10% greater than in the control. The cells show no apparent differences in their morphology. In the 2,090 and 3,200 mg. % concentrations, the extent of cell migration is respectively 16 and 30% less than in the controls, and the cells in each concentration are filled with darker and larger granulations than in the control.

Chicken Blood Leucocytes. Polymorphonuclear Leucocytes. After 24 hours' incubation in 390 and 650 mg. % concentrations, the extent of migration of the polymorphonuclear leucocytes is respectively about 20 and 14% greater than in the controls. This stimulation continues for 6 days. No morphological differences are observed in these cells. In the 1,150, 2,090, and 3,200 mg. % concentrations, the migration is respectively about 6, 4 and 3% more extensive than in the controls. No morphological differences are observed in the 1,150 mg. per % concentration, except that the cells are somewhat granular. In the 2,090 mg. % concentration, the cells appear more densely packed around the central leucocytic fragment than in the control, and are granular. In the 3,200 mg. % concentration, the cells are granular and rounded.

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[4] Yen-lang Huang, *Folia Pharm. Japon.* (Breviaria 143), 1934, **18**, 123; Abstract in *Chem. Abstr.*, 1934, **283**, 7275.

Monocytes. After 24 hours' incubation in the 390, 650, 1,150, and 2,090 mg. % concentrations, cell migration is stimulated, as indicated by a greater density in the cell population, rather than by an increase in the area of the colonies. The cells are active in all concentrations. No morphological differences are observed in the 2 lower concentrations, compared with the controls. In the 2 higher concentrations, the cells appear larger and more coarsely granular. In the 3,200 mg. % concentration, the extent of cell migration is less than in the control. The cells appear coarsely granular and rounded. After 6 days' incubation in the 390 and 650 mg. % concentrations, there is practically no difference in the extent of cell migration as compared with the controls, but the cells are more granular. In the 1,150 mg. % concentration, the monocytes, although granular, are more numerous and active than in the control. In the 2,090 mg. % concentration, the cells seem to have increased in number, are larger in size, and distinctly more granular than in the control. In the 3,200 mg. % concentration, there are many degenerated cells, but a number of large, active macrophages filled with very coarse granulations survive.

Iris Epithelium. In all concentrations of glucose, the rate of migration of the cells is practically the same as in the controls over a period of 9 days. No apparent difference is noted in the cell morphology, except that in the 3,200 mg. % concentration the cells are more granular than in the control or in any of the other concentrations. Although the experiment in sugar concentration was terminated on the 9th day, the cultures were kept and transferred after the 12th day into flasks containing the ordinary nutrient medium. After a few days, new cells started to grow from all the fragments, regardless of the sugar concentration in which they had been cultivated.

Conclusions. Under the conditions of these experiments, fibroblasts from a 24-year-old strain tolerate glucose concentrations of 390, 650, and 1,150 mg. % over a period of 7 days. There is no evidence of an inhibiting or toxic effect. The higher concentrations, namely, 2,090 and 3,200 mg. %, have an inhibiting effect from the very beginning and are ultimately toxic.*

Blood leucocytes are more sensitive and less tolerant to abnormal concentrations of glucose, as shown by the fact that these cells deteriorate more quickly in the higher concentrations of glucose than do fibroblasts and epithelial cells. Migration of the polymorphonuclear cells is stimulated by concentrations of 390, 650, and 1,150 mg. %. These concentrations do not injure the cells. The higher concentrations, although stimulating in the beginning, later become inhibiting and quite toxic. The monocytes appear to be stimulated by glucose in all the concentrations except the very highest. The cells are active in all, but appear coarsely granular in the three highest concentrations. The 3,200 mg. % concentration is, in fact, toxic.*

Iris epithelial cells tolerate each concentration of glucose except that of 3,200 mg. %.* In the latter, the cells become granular. However, after transfer into ordinary nutrient medium, all the cultures recover and grow normally.

8830 C

Reaction of Sensitized Dog's Kidney to Horse Serum Injected into the Renal Artery.

A. E. PARKS AND B. L. TAUSSIG. (Introduced by H. A. Christian.)

From the Medical Clinic, Peter Bent Brigham Hospital, and the Laboratory of the Department of Medicine, Harvard Medical School, Boston, Massachusetts.

The rôle of allergy in the pathogenesis of glomerulo-nephritis has been a subject of experimental investigation for many years and offers an attractive theory of the etiology of this disease. It has long been known that injections of foreign proteins into previously sensitized animals produce lesions in the kidney. Longcope[1] in 1913 showed that animals which were sensitized to horse serum or egg albumin and later given a shock dose of the same protein developed focal renal lesions among which were marked glomerular changes. Duval and Hibbard[2] found that parenteral injections of streptococcus filtrates into animals produced glomerular lesions only if the animals had been previously sensitized to the filtrates. The fact that post-scarlatinal glomerulo-nephritis appears during convalescence rather than at the height of the disease speaks strongly in favor of the "allergic" theory of this disease. Hepler and Simonds[3] studied the effects of injecting proteins directly into the kidneys of sensitized animals. Rabbits were sensitized by subcutaneous injections of horse serum or egg albumin; later, the protein was introduced directly into the kidney through the skin. They observed hemorrhage and necrosis of the injected kidneys and concluded that these changes resulted from an anaphylactic inflammation caused by the direct toxic effect of the antigen on the sensitized tissues (Arthus phenomenon). The lesions thus produced, however, bore no analogy to the histological changes occurring in human glomerulo-nephritis. Masugi,[4] on the other hand, claimed to have produced the picture of a true glomerulo-nephritis by injecting proteins directly into the renal arteries of rabbits which had been previously sensitized. He emphasized the importance of having the foreign protein reach the kidney in a concentrated form, and believed that the unconvincing results of previous workers were due to a failure to bring about the necessary concentration in the glomeruli.

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* It is possible, of course, that this inhibiting effect is due to the reduction in salts that had to be made to keep the solution isotonic. When adding glucose, the solution will be hypertonic unless the salt concentration is lowered. From previous work, it seemed probable that injury to the cells would occur more quickly in hypertonic solutions than in those with reduced salt content. It is interesting to note that Wilmer, using hypertonic solutions and fresh tissue, found approximately the same limits of tolerance as those reported here.

[1] Longcope, W. T., *J. Exp. Med.*, 1913, **18**, 678.

[2] Duval, C. W., and Hibbard, R. J., *J. Exp. Med.*, 1926, **44**, 567.

The work to be described in this paper is an attempt to produce renal lesions in dogs by methods similar to those that Masugi used in rabbits, and then to study the kidneys from both functional and histological aspects.

Method. Nine healthy, well-nourished dogs which were without evidences of previous nephritis were used in these experiments. Two sensitizing injections of 5.0 cc. of horse serum were given intravenously at 2-day intervals to all dogs excepting one which received 7 injections at intervals of 3 days, the doses being 4.0, 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 cc. From 16 to 21 days later about 3.0 cc. of horse serum were injected directly into the left renal artery according to the technique described by Quinby and Fitz[5]. The kidney was approached retroperitoneally and the renal artery and vein were exposed, freed by blunt dissection, and ligatures placed about them. Simple traction on these ligatures sufficed to shut off the flow of blood in the vessels. Both renal vessels were occluded and about 3.0 cc. of horse serum were injected into the artery by means of a syringe fitted with a fine bent needle. The artery was then released to allow the blood to drive the serum into the kidney. After 30 seconds the artery was again occluded, and 10 to 15 cc. of blood were withdrawn from the distended vein by a sharp hypodermic needle attached to a 20 cc. syringe. This blood, presumably, contained most of the injected horse serum. Normal circulation was then allowed to reestablish itself. Pressure of 2 or 3 gauze wipes on the renal vein usually controlled the hemorrhage. With complete hemostasis the wipes were removed and the wound repaired. In 5 of the 9 animals a second shock dose was administered from 12 to 34 days after the first. The method of operation was the same, but increased difficulty was encountered because of adhesions and scar tissue from the first operation. Biopsy specimens were taken from the kidney at the beginning of the experiments and before each arterial injection in 8 out of 9 cases.

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[3] Hepler, O. E., and Simonds, J. P., *Am. J. Path.*, 1929, **5**, 473.

[4] Masugi, M., and Sato, Y., *Virchow's Arch. f. path. Anat.*, 1934, **293**, 615.

[5] Fitz, R., and Quinby, W. C., *Arch. Int. Med.*, 1915, **15**, 303.

After an interval of 1 to 21 days after the final shock dose of horse serum, the dogs were anesthetized with an intravenous injection of 35 mg. of nembutal per kg. body weight, and the ureters exposed through a suprapubic midline incision. Small catheters were inserted into each ureter through a small incision and tied into place. Separate specimens of urine from each kidney were collected into graduated cylinders and the half-hourly output carefully recorded. A satisfactory flow of clear urine from each catheter having been established, 3.0 mg. of phenolsulphonephthalein was injected intravenously, and the excretion from each kidney during the following 2 hours recorded. According to the weight of the dogs, 0.5 to 1.0 cc. of salyrgan was then injected intravenously, and the diuretic response of each kidney was observed at half hour periods. At the end of diuresis the dogs were killed by intravenous chloroform. Throughout the procedure a continuous intravenous flow of physiological saline was maintained. The urine from each kidney was examined for albumin and microscopic sediment, and chloride and creatinine determinations were made on each specimen. After the animals were killed three blocks were taken from each kidney, and together with the biopsy specimens were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Results. All dogs behaved normally after the injections of the sensitizing doses of horse serum. At no time throughout the course of the experiments were there any urinary abnormalities with the exception of occasional slight traces of albumin following the injection of horse serum into the renal artery. There was no constant difference between the secretion of urine from the 2 kidneys following administration of salyrgan, nor were there any differences in the chloride and creatinine excretion. The phenolsulphonephthalein excretion was the same from the 2 kidneys of all dogs excepting 2. The kidneys of these 2, however, showed gross and microscopic abnormalities which could not possibly be explained on an allergic basis. The left kidney of one presented the appearance of multiple infarction due in all likelihood to the presence of particulate matter contained in the serum. This particulate matter was noted at the time of injection into the renal artery. That of the other contained numerous microscopic abscesses caused, no doubt, by infection introduced at the time of injection. The kidneys of 5 of the 9 dogs were entirely normal both grossly and microscopically. All biopsies showed normal kidney tissue. The kidneys of 2 of the dogs showed changes which will be described and discussed below.

Dog No. 1, a male weighing 10 kilograms, was given two intravenous sensitizing injections of 5.0 cc. of horse serum, and 21 days later 5.0 cc. were injected into the left renal artery. On the following day functional studies revealed no impairment of either kidney and the animal was killed for autopsy. With the exception of the left kidney all the organs were normal. The kidneys were of equal size and of normal reddish-brown color, but scattered over the surface of the left kidney were numerous small pin-point whitish depressed areas surrounded by rings of hyperemia. The cut surface was not abnormal. The microscopic appearance was that of exudative rather than proliferative lesions. The glomerular tufts were swollen and somewhat more cellular than normal and tended to fill the capsular spaces. There was some increase in the sub-endothelial ground substance and slight proliferation of the endothelial cells. There was no increase in interstitial tissue. There was an albuminous exudate in the capsular spaces, but no inflammatory or red blood cells. The tubules showed slight cloudy swelling of the epithelium and an albuminous exudate in the lumina. The vessels were normal. The right kidney showed similar but less marked lesions.

Dog No. 2 was a male weighing 9.6 kilograms. Horse serum was injected intravenously as in the case of Dog No. 1. Twenty-one days later, horse serum was injected into the renal artery. After 6 days, routine functional studies were made, and the dog was killed. The microscopic appearance of the biopsy specimens and the functional behavior and gross appearances of both kidneys were entirely normal. Microscopically, the glomerular tufts showed little or no proliferative change, but the epithelium of the Bowman's capsule was often seen to be thickened; the capsular spaces contained much albuminous material and red blood cells. The tubular lumina were filled with an albuminous exudate, but the tubules themselves, as well as the interstitial tissues and blood vessels, were normal. The right kidney showed similar but somewhat less marked changes.

Summary. In a series of 9 dogs, an attempt was made to produce an experimental glomerulo-nephritis by the injection of horse serum into the left renal artery of previously sensitized animals. Functional studies failed to reveal any impairment as a result of the procedure, and pathologically no animal showed a real glomerulo-nephritis. In the kidneys of 2 dogs lesions were found which were the result of traumatization and infection respectively; in 2, minor changes were noted, as just described, whose relation to the procedure of the experiment is uncertain; in 5 no pathological changes were observed. It is concluded that the procedure described by Masugi as productive of glomerulo-nephritis in rabbits failed to produce the disease in dogs.

8831 P

Effects of Increased Metabolism on the Ketone Body Excretion of Depancreatized Dogs.*

S. B. BARKER. (Introduced by William H. Chambers.)

From the Department of Physiology, Cornell University Medical College, New York City.

Since fat is a source of ketone bodies, increasing the amounts of this foodstuff oxidized by completely diabetic organisms should produce an increase in the ketone body excretion. According to Shaffer's quantitative expression, every mol of fatty acid oxidized without the simultaneous combustion of antiketogenic material yields one mol of acetoacetic acid. The evidence from a variety of sources indicates that the depancreatized animal depends largely upon fat to furnish its bodily energy, both at rest and during activity,[1] and, furthermore, that it is not able to oxidize significant amounts of

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* Abstract of a thesis presented to the faculty of the Graduate School of Cornell University for the degree of Doctor of Philosophy, June, 1936.

[1] Chambers, W. H., Kennard, M. A., Pollack, H., and Dann, M., *J. Biol. Chem.*, 1932, **97**, 525.

TABLE I.

Summary of Changes in Metabolism of Depancreatized Dogs Produced by Exercise and by Dinitrophenol.

Exp. (1)	Dog No. (2)	Basal		R.Q.		Total Increased		Expected acetone,* mg. (9)	% Obtained (10)	Expected acetone,† mg. (11)	% Obtained (12)
		Calories /hour (3)	Total acetone, mg./hr. (4)	Basal (5)	Exp. (6)	Cal. (7)	Acetone, mg. (8)				
Exercise	B-3	22.7	200	.73	.72	10.1	—39	490	‡	89	‡
	5	24.3	66	.72	.72	19.0	44	921	5	52	85
	11	28.9	47	.71	.70	14.2	46	688	7	23	200
	13	31.4	118	.70	.69	6.4	237	310	76	24	988
	14	28.4	266	.73	.71	27.4	—202	1327	‡	256	‡
	18	28.1	48	.70	.71	41.8	25	2015	1	71	35
D.N.P.	6	28.4	315	.71	.73	134.8	—174	6520	‡	1494	‡
	11	15.3	36	.71	.71	4.9	0	237	0	12	0
	15	13.5	118	.71	.71	45.2	—62	2188	‡	396	‡
	16	22.7	45	.72	.71	68.9	35	3337	1	137	26
	17	18.3	23	.74	.71	82.1	—36	3978	‡	103	‡
	18	37.3	29	.72	.72	301.0	—23	14580	‡	234	‡

*Calculated from increased fat oxidized. See text.

†Calculated from basal metabolism and ketosis. See text.

‡Acetone excretion decreased.

antiketogenic material.[2] The general purpose of this research was to investigate acetone body formation in the depancreatized dog by increasing the burning of fat.

The methods used to stimulate oxygen consumption were exercise, injection of dinitrophenol (D.N.P.), and administration of desiccated thyroid gland.† An open-circuit respiration set-up was employed for the metabolism determinations, using a Carpenter-Haldane apparatus for the gas analyses. For the assay of urinary constituents, standard techniques were used, and in addition the amount of expired air acetone was determined.[3] All parts of the work were rigidly controlled.

Exercise. Eighteen experiments, including basal, exercise, and recovery periods, were performed on 6 depancreatized dogs. The respiratory quotients of exercise plus recovery (column 6, Table I) show no significant changes from the basal quotients (column 5), indicating that fat furnished the fuel for the increased metabolism. As a basis for comparisons, the total increases in ketosis and in caloric output during each experiment are calculated for the exercise plus the recovery periods. The amounts of additional ketone substances to be expected are computed in two different manners, the first from the increased amounts of fat oxidized (column 9), and the second from the basal proportions of acetone excretion and heat production (column 11). Using a heat value of 7.44 calories and an acetone yield of 0.36 gm. per gm. of fat oxidized, one should expect 0.36/7.44 gm. of extra ketones for each additional calorie of heat produced. Compared with the theoretical values, the amounts obtained were all less than 35% of the quantity expected, with one exception of 76%.

The second method of calculation is outlined as follows:

$$\frac{\text{Total increased calories}}{\text{Basal calories}} \times \text{Basal acetone} = \text{Expected increase in acetone}$$

When these values are used as standards, the amounts of extra acetone to be expected are considerably lowered, as can be seen from the table. Of the 18 sets of results, 4 showed decreased excretion of acetone bodies, while in 14 the amounts were increased over the respective basals. Five of these 14 were grouped about 30% of the theoretical, while the remainder were spread out quite evenly up to 200%, with one high value of 988%. There were only 2 figures between 80 and 120%, the range which one would consider "quantitative". The wide distribution of the percentages obtained by this second method of calculation does not indicate any quantitative relationship between the increased fat oxidation and changes in the excretion of acetone bodies.

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[2] Cori, C. F., *Physiol. Rev.*, 1931, **11**, 143.

† We wish to express our thanks for the thyroid preparation supplied by the Burroughs, Wellcome Company.

[3] Barker, S. B., *Am. J. Physiol.*, 1936, **116**, 5.

Dinitrophenol. The acetone changes following D.N.P. stimulation of metabolism proved to be even more startling, as in no instance did the excretion of the four-carbon compounds increase significantly. In 9 of 12 experiments the ketosis did not increase at all, or fell, in spite of increases of metabolism in these cases from 5 to 300 calories. When the expected additional ketones are computed from the extra fat burned following D.N.P. stimulation of heat output, the percentages obtained are all less than 1%. Recalculated from the basal metabolism and ketosis, the highest amount then obtained is 26%, with the other values being 20% and 2%, respectively. The amounts to be expected from the fat burned range from 200 to 14,000 mg., while those by the second method of calculation are much lower, being 5 to 1490 mg.

Thyroid. The administration of desiccated thyroid substance produced a much more prolonged elevation of metabolism than could be obtained with either of the other 2 methods. Four dogs were the subjects for 5 experiments, each consisting of a series of basal metabolism determinations during 3 days without thyroid and during 3 corresponding days following prolonged feeding of glandular substance.

Three of the dogs showed no increase in acetone body production in the hyperthyroid periods, while the other, No. B-16, excreted 2 to 3 times the amount of its basal ketosis in both of 2 hyperthyroid series. The effects of the ingestion of meat were studied on 3 of the animals used, and it was found that this foodstuff produced a marked increase in ketone body elimination of dog No. B-16, but only very slight rises in the amounts excreted by the other 2. It should be noted that the differences in these changes were quite similar to those occurring after thyroid administration. However, in the entire set of experiments, the protein catabolism was heightened only once by the thyroid treatment—in 1 of the 2 series on No. B-16, which produced increased ketosis.

Summary. The resting metabolism of depancreatized dogs was raised by exercise, by dinitrophenol injection, and by thyroid administration, always, as far as could be learned, at the expense of increased oxidation of fat. Out of the total of 35 series of experiments, none showed increased ketosis commensurate with the increased amounts of fat burned. The quantities of extra acetone to be expected were considerably lower when these were calculated from the basal heat and acetone values rather than from the increased fat oxidation. Even computed in this manner, however, there were only a few of the exercise and thyroid, and none of the D.N.P., results which were in agreement with the theoretical ketone excretion.

SECRETARY'S ANNUAL REPORT

April 1, 1935 to April 1, 1936

COUNCIL MATTERS

The PROCEEDINGS are now issued in two volumes a year. The present year's volumes contained 1586 pages. Members are charged $4.00 a year and for excess space 60 percent below cost. Our printing costs are lower than any other comparable journal.

To meet the growing costs, chiefly due to enlargement, the Council decided not to raise members' dues, but to use the interest from the Endowment and Surplus Funds.

To meet the greater volume of manuscripts submitted and the greater standards of acceptance, the Board of Editors was increased to 14 (see list below).

There has been, in a few instances, commercialism of articles in the PROCEEDINGS. The Council has therefore adopted the following:

> *"The* PROCEEDINGS *are intended for publication of scientific manuscripts for scientific workers and not as a vehicle for advertising. It is understood that the author of each manuscript agrees to discourage the circulation of his reprints for advertising or commercial purposes. Such reprints are intended for research workers in related fields. Reprints are limited to 500. The* PROCEEDINGS *are published under the copyright law.*

The Council authorized the incorporation of the Society.

The revised Constitution and By-Laws of the Society was almost unanimously approved by the members.

The Council decided not to approve the formation of new sections outside the United States and Canada and that foreign investigators may be elected to membership as in the past.

The PROCEEDINGS (from April 1935 to April 1936) contains 657 manuscripts 49% of which were preliminary, 51%, complete.

MEMBERSHIP

Members, March 31, 1935	1257	
Elected during year	88	
Honorary Members	7	
Total		1352
Resignations	9	
Deaths	13	
Arrears	5	27
Total Membership, March 31, 1936		1325

	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936
Membership	779	851	936	986	1026	1062	1138	1146	1218	1266	1325

SUBSCRIPTIONS

Subscriptions, March 31, 1935	449
Subscriptions, March 31, 1936	506
Total No. of PROCEEDINGS distributed 3/31/35	1715
Total No. of PROCEEDINGS distributed 3/31/36	1831

OFFICERS elected April 15, 1936

President: P. E. Smith.
Vice-President: E. F. DuBois.
Secretary-Treasurer: A. J. Goldforb

Council: R. Chambers
H. S. Gasser

H. W. Smith
W. B. Cannon } Hold over
F. R. Sabin

Past Presidents (by constitution): E. B. Wilson, S. Flexner, F. S. Lee, T. H. Morgan, J. Ewing, W. J. Gies, G. Calkins, G. B. Wallace, J. W. Jobling, S. R. Benedict, P. Rous, D. J. Edwards, A. R. Dochez, E. L. Opie.

Chairmen of Sections (See Sections).

Nominating Committee (elected April 15, 1936): A. C. Ivy, Chairman, G. Baehr, R. K. Cannan, E. V. Cowdry, F. C. Koch, W. J. MacNeal, T. M. Rivers, C. Voegtlin, S. B. Wolbach.

Editorial Committee (appointed by Council): D. W. Bronk, G. W. Corner, A. B. Hastings, S. B. Hooker, A. C. Ivy, C. A. Kofoid, H. B. Lewis, A. B. Luckhardt, W. S. McCann, W. deB. MacNider, A. M. Pappenheimer, T. M. Rivers, H. B. Williams, A. J. Goldforb.

Membership Committee (appointed by Sections): R. Ashman, E. R. Becker, W. R. Bloor, E. A. Doisy, H. Goldblatt, I. Greenwald, P. J. Hanzlik, A. C. Ivy, W. P. Larson, A. L. Tatum, C. H. Thienes.

Financial Committee: W. J. Gies, W. H. Park, A. J. Goldforb.

PAST OFFICERS

Date	*President*	*Vice-President*	*Secretary*	*Treasurer*
1903-04	S. J. Meltzer	W. H. Park	W. J. Gies	G. N. Calkins
1904-05	S. J. Meltzer	J. Ewing	W. J. Gies	G. N. Calkins
1905-06	E. B. Wilson	E. K. Dunham	W. J. Gies	G. N. Calkins
1906-07	S. Flexner	E. K. Dunham	W. J. Gies	G. N. Calkins
1907-08	S. Flexner	T. H. Morgan	W. J. Gies	G. N. Calkins
1908-09	F. S. Lee	T. H. Morgan	W. J. Gies	G. Lusk
1909-10	F. S. Lee	W. J. Gies	E. L. Opie	G. Lusk
1910-11	T. H. Morgan	W. J. Gies	E. L. Opie	G. Lusk
1911-12	T. H. Morgan	P. A. Levene	G. B. Wallace	G. Lusk
1912-13	J. Ewing	P. A. Levene	G. B. Wallace	C. Norris
1913-14	J. Ewing	C. W. Field	H. C. Jackson	C. Norris
1914-15	G. Lusk	W. J. Gies	H. C. Jackson	J. R. Murlin
1915-16	G. Lusk	G. N. Calkins	H. C. Jackson	
1916-17	J. Loeb	W. J. Gies	H. C. Jackson	

1917-18	W. J. Gies	J. Auer	H. C. Jackson
1918-19	W. J. Gies	J. Auer	H. C. Jackson
1919-20	G. N. Calkins	G. B. Wallace	H. C. Jackson
1920-21	G. N. Calkins	G. B. Wallace	H. C. Jackson
1921-22	G. B. Wallace	J. W. Jobling	H. C. Jackson
1922-23	G. B. Wallace	J. W. Jobling	H. C. Jackson
1923-24	H. C. Jackson	J. W. Jobling	V. C. Myers
1924-25	H. C. Jackson	J. W. Jobling	A. J. Goldforb
1925-26	J. W. Jobling	S. R. Benedict	A. J. Goldforb
1926-27	J. W. Jobling	S. R. Benedict	A. J. Goldforb
1927-28	S. R. Benedict	P. Rous	A. J. Goldforb
1928-29	S. R. Benedict	P. Rous	A. J. Goldforb
1929-30	P. Rous	D. Marine	A. J. Goldforb
1930-31	P. Rous	D. J. Edwards	A. J. Goldforb
1931-32	D. J. Edwards	A. R. Dochez	A. J. Goldforb
1932-33	A. R. Dochez	E. L. Opie	A. J. Goldforb
1933-34	A. R. Dochez	E. L. Opie	A. J. Goldforb
1934-35	E. L. Opie	P. E. Smith	A. J. Goldforb
1935-36	E. L. Opie	P. E. Smith	A. J. Goldforb

SECTIONS

Cleveland, Ohio

Chairman: J. T. Wearn. Secretary: E. Muntwyler. Members: 39.
Meetings: Western Reserve University, December 13, 1935.

District of Columbia

Chairman: J. A. Hawkins. Secretary: W. C. Hess. Members: 25.
Meetings: George Washington University, November 19, 1935.

Illinois

Chairman: M. B. Visscher. Secretary: L. A. Crandall. Members: 116.
Meetings: University of Illinois Medical School, November 26, 1935.
Northwestern University Medical School, February 18, 1936.
Loyola University Medical School, April 21, 1936.
University of Chicago Medical School, May 26, 1936.

Iowa

Chairman: J. A. Greene. Secretary: C. P. Berg. Members: 34.
Meetings: State University of Iowa, November 5, 1935.
Grinnell College, December 3, 1935.
State University of Iowa, February 6, 1936.
State University of Iowa, March 3, 1936.
State University of Iowa, May 5, 1936.

Minnesota

Chairman: F. H. Scott. Secretary: F. H. Scott. Members: 45.
Meetings: University of Minnesota, November 20, 1935.
University of Minnesota, March 18, 1936.
University of Minnesota, May 20, 1936.

Missouri

Chairman: E. A. Doisy. Secretary: G. H. Bishop. Members: 49.
Meetings: St. Louis University Medical School, October 9, 1935.
Municipal Auditorium, St. Louis, November 19, 1935.
Washington University Medical School, February 12, 1936.
St. Louis University Medical School, May 13, 1936.

New York

Chairman: P. E. Smith. Secretary: A. J. Goldforb. Members: 731.
Meetings: New York Academy of Medicine, October 16, 1935.
New York Academy of Medicine, November 20, 1935.
New York Academy of Medicine, December 18, 1935.
New York Academy of Medicine, January 15, 1936.
New York Academy of Medicine, February 19, 1936.
New York Academy of Medicine, March 18, 1936.
New York Academy of Medicine, April 15, 1936.
New York Academy of Medicine, May 20, 1936.

Pacific Coast

Chairman: C. D. Leake. Secretary: J. Field, II. Members: 89.
Meetings: University of California at Los Angeles, June 27, 1935.
Stanford University, October 19, 1935.
University of California Hospital, December 4, 1935.
Stanford University Medical School, February 5, 1936.
Mount Zion Hospital, San Francisco, March 11, 1936.
University of California, Berkeley, May 4, 1936.

Peiping (China)

Chairman: C. N. Frazier. Secretary: H. C. Chang. Members: 36.
Meetings: Peiping Union Medical College, October 23, 1935.
Peiping Union Medical College, February 19, 1936.
Peiping Union Medical College, April 22, 1936.

Southern

Chairman: Roy H. Turner. Secretary: H. M. Mayerson. Members: 37.
Meetings: Municipal Auditorium, St. Louis, November 19, 1935.
Tulane University, February 26, 1936.
Tulane University, May 22, 1936.

Southern California

Chairman: D. R. Drury. Secretary: G. H. Ball. Members: 32.
Meetings: University of California at Los Angeles, June 27, 1935.
Pomona College, January 25, 1936.
University of California at Los Angeles, May 5, 1936.

Western New York

Chairman: J. A. Dye. Secretary: G. H. Maughan. Members: 53.
Meetings: University of Buffalo, December 14, 1935.
University of Rochester, February 25, 1936.

Wisconsin

Chairman: E. L. Sevringhaus. Secretary: M. R. Irwin. Members: 32.

DEATHS OF MEMBERS

The Council records with regret the deaths of the following members: Doctors A. C. Abbott, H. M. Adler, W. C. Austin, C. R. Bardeen, W. T. Councilman, H. B. Eisberg, R. Erdmann, R. G. Harris, F. S. Jones, J. J. R. MacLeod, L. B. Mendel, C. Norris, E. T. Reichert, M. A. Rothschild.

A. J. GOLDFORB,
Secretary

TREASURER'S REPORT

April 1, 1935—April 1, 1936

RECEIPTS

Balance on Hand, April 1, 1935		$ 5,013.78
Income, 1935-36:		
Dues	$ 5,243.80	
Reprints	2,948.55	
Excess Space	1,627.60	
Cuts	536.14	
Changes	55.51	
Subscriptions	3,247.76	
Back Numbers Sold	315.71	
Miscellaneous	.25	
Total Annual Income		$13,975.32
Total Cash Available, April 1, 1935-April 1, 1936		$18,989.10

DISBURSEMENTS

Publication Cost of Proceedings:		
Printing	$ 6,871.63	
Reprints	2,963.07	
Cuts	586.55	
		$10,421.25
Administrative Expenses:		
Office Supplies, Equipment, Postage, Telephone	$ 442.64	
Salaries	2,020.00	
Storage and Insurance	43.00	
Miscellaneous	64.04	
		$ 2,569.68
Total Annual Disbursements		$12,990.93
Transferred to Endowment Fund		2.00
Cash Balance, April 1, 1936		5,996.17
Total		$18,989.10

SUMMARY

Income (net)	$13,975.32
Disbursements (net)	12,990.93
Surplus for year	$ 984.39

Bills payable:	
Printing	$484.61
Reprints	186.47
Cuts	25.91
	$696.99
Bills receivable	$680.25

FUNDS

Endowment Fund

April 1, 1935	$14,855.50	
Received during year	2.00	
Interest to April 1, 1936	533.42	
Total		$15,390.92
Invested in New York Title and Mort. Co.	$ 6,000.00	
Title Guarantee and Trust	2,500.00	
Lawyers Mortgage Co.	1,500.00	
Railroad Federal Savings	24.17	
Bowery Savings Bank	1,353.29	
Industrial Bonds (purchase price) Present value $4,197.50	4,013.46	
Total		$15,390.92

Surplus Fund

April 1, 1935	$ 9,324.86	
Interest to April 1, 1936	368.86	
Total		$ 9,693.72
Invested in Title Guarantee and Trust	$ 2,900.00	
Railroad Federal Savings	3,036.87	
Bowery Savings Bank	583.38	
Industrial Bonds (purchase price) Present value $3,395.00	3,173.47	
Total		$ 9,693.72

Life Membership Fund

Invested in Railroad Federal Savings and Loan	$ 75.00

Auditors' Report

April 8, 1936

We have examined the Treasurer's report and have verified the statements therein against the books of the Society. We are satisfied that the records of financial transactions are in good order and accurately kept.

(Signed) GEORGE BAEHR
EUGENE F. DUBOIS
R. K. CANNAN.

MEMBERS' LIST

Honorary Members

Flexner, Simon	Rockefeller Institute
Howell, William H.	Johns Hopkins Univ.
Lombard, Warren P.	Univ. of Michigan
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Porter, William	Harvard Univ.
Richet, Charles	Paris, France
Von Muller, Friedrich	Munich, Germany
Wilson, Edmund B.	Columbia Univ.

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Adams, William E.	Univ. of Chicago
Addis, Thomas	Med., Stanford Univ.
Adolph, E. F.	Univ. of Rochester Med.
Adolph, W. H.	Peiping, China
Alexander, Harry L.	Washington Univ.
Allen, Bennet M.	Univ. of California
Allen, Edgar	Yale Univ.
Allen, Ezra	N. Y. Homeopathic Med.
Allen, William F.	Univ. of Oregon
Alles, G. A.	Los Angeles, California
Alsberg, Carl L.	Stanford Univ.
Alt, Howard L.	Northwestern Univ. Med.
Althausen, T. L.	Univ. of California Med.
Alvarez, Walter C.	Mayo Clinic
Alving, A. S.	Univ. of Chicago
Amberg, Samuel	Mayo Clinic
Amberson, W. R.	Univ. of Tennessee Med.
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Andersen, Dorothy H.	Coll. of Physicians and Surgeons
Anderson, H. H.	Univ. of California Med.
Anderson, John E.	Univ. of Minnesota
Anderson, John F.	E. R. Squibb & Son
Anderson, Rudolph J.	Yale Univ.
Anderson, William E.	Yale Univ.
Andrews, Edmund	Univ. of Chicago
Anson, Mortimer L.	Rockefeller Institute, Princeton, N. J.
Arnold, Lloyd	Univ. of Illinois
Aronson, J. D.	Henry Phipps Institute
Asdell, S. A.	Cornell Univ.

*Deceased.

Asher, Leon	Berne, Switzerland
Ashman, Richard	Louisiana State Univ. Medical Center
Asmundson, V. S.	Univ. of California
Atchley, D. W.	Presbyterian Hospital, New York City
Atwell, Wayne J.	Univ. of Buffalo, N. Y.
Aub, Joseph C.	Huntington Memorial Hospital, Boston
Auer, John	St. Louis Univ.
Austin, J. Harold	Univ. of Pennsylvania
Avery, O. T.	Rockefeller Institute, N. Y. City
Babkin, Boris P.	McGill Univ.
Bachem, Albert	Univ. of Illinois Medical Coll.
Baehr, George	Mt. Sinai Hospital, N. Y. City
Bagg, Halsey J.	Memorial Hospital, N. Y. City
Bailey, Cameron V.	N. Y. Post-Graduate Med.
Baitsell, George A.	Yale Univ.
Bakwin, Harry	N. Y. Univ. and Bellevue Medical Coll.
Baldwin, Francis M.	Univ. of S. Calif.
Baldwin, W. Manning	Albany Medical Coll.
Ball, G. H.	Univ. of California, Los Angeles
Balls, A. K.	U. S. Dept. of Agriculture
Banta, A. M.	Brown Univ.
Banting, Frederick G.	Univ. of Toronto
Barach, Alvin L.	Coll. of Physicians and Surgeons
Barber, W. Howard	New York Univ. Med.
Barbour, Henry G.	Yale Univ.
Barer, Adelaide P.	State Univ. of Iowa
Barnett, George D.	Stanford Univ.
Barr, David P.	Washington Univ.
Barron, E. S. G.	Univ. of Chicago
Barth, L. G.	Columbia Univ.
Bass, Charles	Tulane Univ.
Bast, T. H.	Univ. of Wisconsin
Bates, R. W.	Carnegie Institution of Washington
Bauer, J. H.	Rockefeller Institute
Bauman, Louis	Presbyterian Hospital, N. Y. City
Baumann, E. J.	Montefiore Hospital, N. Y. City
Baumberger, J. Percy	Stanford Univ.
Bayne-Jones, S.	Yale Univ.
Bazett, H. C.	Univ. of Pennsylvania
Bean, John W.	Univ. of Michigan
Beard, H. H.	Louisiana State Univ. Medical Center
Beard, J. W.	Rockefeller Institute
Beck, Claude S.	Western Reserve Univ.
Becker, E. R.	Iowa State Coll.
Beckman, Harry	Marquette Univ. Med.
Beckwith, T. D.	Univ. of California, Los Angeles
Behre, Jeannette A.	Cornell Univ. Medical Coll.
Belding, David L.	Boston Univ.
Bellamy, Arthur W.	Univ. of California, Los Angeles

Benedict, S. R.	Cornell Univ. Medical Coll., N. Y. City
Berg, B. N.	Columbia Univ.
Berg, C. P.	State Univ. of Iowa
Berg, William N.	New York City
Bergeim, Olaf	Univ. of Illinois
Bergey, David H.	National Drug Co., Philadelphia
Bergmann, Max	Rockefeller Institute
Bernhard, Adolph	Lenox Hill Hospital, N. Y. City
Bernthal, T. G.	Univ. of Michigan
Berry, George P.	Univ. of Rochester Med.
Beutner, R.	Univ. of Louisville
Bieter, Raymond N.	Univ. of Minnesota
Bills, C. E.	Mead, Johnson and Co., Evansville, Ind.
Binger, Carl A. L.	Rockefeller Institute, N. Y. City
Birkhaug, Konrad E.	Geophysic Institute, Bergen, Norway
Bishop, George H.	Webster Groves, Mo.
Bishop, Katharine S.	Univ. of California
Blackfan, K. D.	Harvard Med.
Blair, John E.	Hospital for Joint Diseases, N. Y. City
Blake, F. G.	Yale Univ.
Blakeslee, Albert F.	Station for Exp. Evolution, Cold Spring Harbor, N. Y.
Blalock, Alfred	Vanderbilt Univ. Med.
Blatherwick, Norman R.	Metropolitan Life Insurance Co., N. Y. City
Blau, Nathan F.	Cornell Univ. Medical Coll.
Blinks, L. R.	Stanford Univ.
Bliss, Sidney	Tulane Univ.
Bloch, Robert G.	Univ. of Chicago
Block, Richard J.	N. Y. State Psychiatric Institute
Bloom, William	Univ. of Chicago
Bloomfield, A. L.	Stanford Univ. Med.
Bloor, W. R.	Univ. of Rochester
Blount, R. F.	Univ. of Minnesota
Blum, Harold F.	Univ. of California
Blumgart, H. L.	Beth Israel Hospital, Boston
Bodansky, A.	Hospital for Joint Diseases, N. Y. City
Bodansky, Meyer	John Sealy Hospital, Galveston, Texas
Bodine, J. H.	State Univ. of Iowa
Bodo, Richard	N. Y. Univ. and Bellevue Medical Coll.
Bollman, Jesse L.	Mayo Clinic
Boor, Alden K.	Univ. of Chicago
Boothby, Walter M.	Kahler Hospital, Rochester, Minn.
Boots, Ralph H.	Presbyterian Hospital, N. Y. City
Borsook, Henry	California Institute of Technology
Bowen, B. D.	Buffalo General Hospital
Boyd, Theo. E.	Loyola Univ.
Boyden, E. A.	Univ. of Minnesota Med.
Bradford, William L.	Univ. of Rochester
Bradley, H. C.	Univ. of Wisconsin
Brand, Erwin	N. Y. State Psychiatric Institute
Branham, Sara E.	National Inst. of Health, Washington

Brewer, Robert K.	Syracuse Univ.
Briggs, A. P.	St. Louis Univ.
Brodie, Maurice	Dept. of Health, N. Y. City
Bronfenbrenner, J.	Washington Univ.
Bronk, D. W.	Univ. of Pennsylvania
Brooks, Clyde	Louisiana State Univ. Medical Center
Brooks, Matilda M.	Univ. of California
Brooks, S. C.	Univ. of California
Broun, G. O.	St. Louis Univ. Med.
Brown, E. D.	Univ. of Minnesota
Brown, J. Howard	Johns Hopkins Univ.
Brown, John B.	Ohio State Univ.
Brown, L. A.	Transylvania Coll., Lexington, Ky.
Brown, Rachel	N. Y. State Dept. of Health
Brown, Wade H.	Rockefeller Institute, N. Y. City
Bruger, Maurice	N. Y. Post-Graduate Med.
Buchanan, Robert E.	Iowa State Coll.
Buchbinder, W. C.	Michael Reese Hospital, Chicago
Buell, Mary V.	Johns Hopkins Univ.
Bulger, H. A.	Washington Univ.
Bullowa, J. G. M.	Harlem Hospital, N. Y. City
Bunting, C. H.	Univ. of Wisconsin
Bunting, R. W.	Univ. of Michigan
Burch, John C.	Vanderbilt Univ. Med.
Burget, G. E.	Univ. of Oregon Med.
Burky, Earl L.	Johns Hopkins Hospital
Burns, Robert K., Jr.	Univ. of Rochester
Burr, George O.	Univ. of Minnesota
Burr, Harold S.	Yale Univ.
Burrows, M. T.	Pasadena, Calif.
Byerly, T. C.	U. S. Animal Exp. Farm, Beltsville, Md.
Byrne, Joseph	Fordham Univ.

Calkins, Gary N.	Columbia Univ.
Cameron, A. T.	Univ. of Manitoba
Cannan, Robert K.	N. Y. Univ. and Bellevue Medical Coll.
Cannon, Paul R.	Univ. of Chicago
Cannon, Walter B.	Harvard Med.
Cantarow, Abraham	Jefferson Medical Coll.
Carey, E. J.	Marquette Univ. Med.
Carlson, A. J.	Univ. of Chicago
Carmichael, E. B.	Univ. of Alabama Med.
Carruthers, Albert	Peiping Union Medical Coll.
Carter, Edward P.	Johns Hopkins Hospital
Casey, Albert E.	Univ. of Virginia
Cash, James R.	Univ. of Virginia
Casida, L. E.	Univ. of Wisconsin
Cattell, McKeen	Cornell Univ. Medical Coll.
Caulfeild, A. H. W.	Univ. of Toronto
Cecil, R. L.	Cornell Univ. Medical Coll.
Cerecedo, L. R.	Fordham Univ.

Chace, Arthur F.	N. Y. Post-Graduate Medical Coll.
Chaikoff, I. L.	Univ. of California
Chambers, Robert	New York Univ.
Chambers, William H.	Cornell Univ. Medical Coll.
Chang, Hsi Chun	Peiping Union Medical Coll.
Chang, Hsiao-Chien	Peiping Union Medical Coll.
Charipper, H. A.	New York Univ.
Cheer, S. N.	Central Hospital, Nanking, China
Chen, K. K.	Eli Lilly and Co., Indianapolis
Chen, T. T.	Peiping Union Medical Coll.
Cheney, R. H.	Long Island Univ.
Chidester, F. E.	Univ. of Michigan
Child, C. M.	Univ. of Chicago
Chittenden, R. H.	Yale Univ.
Christensen, K.	St. Louis Univ.
Christian, Henry A.	Peter Bent Brigham Hospital
Christman, Adam A.	Univ. of Michigan
Chu, F. T.	Peiping Union Medical Coll.
Clark, A. J.	University of Edinburgh, Scotland
Clark, Guy W.	Lederle Lab., Pearl River, N. Y.
Clark, P. F.	Univ. of Wisconsin
Clarke, Hans T.	Coll. of Physicians and Surgeons
Claude, A.	Rockefeller Inst.
Claussen, S. W.	Strong Memorial Hospital, Rochester, N. Y.
Clawson, Benjamin J.	Univ. of Minnesota
Clifton, Charles E.	Stanford Univ.
Clowes, G. H. A.	Eli Lilly and Co., Indianapolis, Indiana
Coca, A. F.	Oradell, N. J.
Coggeshall, L. T.	Rockefeller Foundation
Coghill, G. E.	Wistar Institute
Cohen, Barnett	Johns Hopkins Med.
Cohen, Martin	N. Y. Post-Graduate Med.
Cohen, Milton B.	St. Alexis Hospital, Cleveland
Cohn, A. E.	Rockefeller Institute, N. Y. City
Cohn, Isidore	New Orleans, La.
Cole, Arthur G.	Univ. of Illinois Med.
Cole, E. C.	Williams Coll.
Cole, Harold H.	Univ. of California, Davis
Cole, L. J.	Univ. of Wisconsin
Cole, Rufus I.	Rockefeller Institute, N. Y. City
Cole, William H.	Rutgers Univ.
Coleman, Warren	New York Univ. Medical Coll.
Collens, William S.	Brooklyn, N. Y.
Collett, Mary E.	Western Reserve Univ.
Collier, William D.	St. Louis Univ.
Collins, H. H.	Crafton, Pa.
Collip, J. B.	McGill University
Conklin, E. G.	Princeton Univ.
Connor, Charles L.	Univ. of California Med.
Cook, Donald H.	School of Tropical Medicine, San Juan, P. R.

Cook, S. F.	Univ. of California
Cooke, J. V.	Washington Univ.
Coombs, Helen C.	N. Y. Homeopathic Medical Coll.
Cope, O. M.	N. Y. Homeopathic Med.
Copenhaver, W. M.	Columbia Univ.
Corey, E. L.	Univ. of Va.
Cori, Carl F.	Washington Univ.
Corley, Ralph C.	Purdue Univ.
Corner, George W.	Univ. of Rochester
Corper, H. J.	National Jewish Hospital, Denver, Colo.
Cotui, F. W.	N. Y. Univ. Medical Coll.
Coulter, Calvin B.	Columbia Univ.
Cowdry, E. V.	Washington Univ.
Cowgill, George R.	Yale Univ.
Cowie, D. M.	Univ. of Michigan
Cox, Warren M., Jr.	Mead Johnson Co.
Craig, C. F.	Tulane Univ.
Crampton, C. Ward	N. Y. Post-Graduate Med.
Crandall, L. A., Jr.	Northwestern Univ.
Crile, George W.	Western Reserve Univ.
Crohn, Burrill B.	Mt. Sinai Hospital, N. Y. City
Cruickshank, E. W. H.	Marischal Coll., Aberdeen
Csonka, F. A.	U. S. Dept. of Agriculture, Washington, D. C.
Cullen, Glenn E.	Children's Hospital, Cincinnati, O.
Cummins, Harold	Tulane Univ.
Cunningham, R. S.	Vanderbilt Univ.
Curtis, G. M.	Ohio State Univ.
Curtis, Maynie R.	Columbia Univ.
Cushing, Harvey W.	Yale Univ.
Cutler, Elliott C.	Peter Bent Brigham Hospital, Boston
Dack, Gail M.	Univ. of Chicago
Dakin, H. D.	Ossining, N. Y.
D'Amour, F. E.	Univ. of Denver
Danforth, Charles H.	Stanford Univ.
Daniel, J. Frank	Univ. of California
Daniels, Amy L.	Univ. of Iowa
Danzer, Charles S.	N. Y. Homeopathic Medical Coll.
Dautrebande, Lucien	Univ. of Liege, Belgium
Davenport, C. B.	Sta. for Exp. Evolution, Cold Spring Harbor, N. Y.
Davenport, H. A.	Northwestern Univ. Med.
Davis, D. J.	Wilmette, Illinois
Davis, M. E.	Univ. of Chicago
Dawson, James A.	Coll. of the City of N. Y.
Dawson, M. H.	Columbia Univ.
Dawson, W. T.	Univ. of Texas Med.
Day, A. A.	Northwestern Univ. Med.
DeEds, Floyd	Stanford Univ. Med.
DeGraff, A. C.	N. Y. Univ. Med.
Dennis, E. W.	Amer. Univ. of Beirut

DeRenyi, G. S.	Univ. of Pennsylvania
Derick, C. L.	Peter Bent Brigham Hospital, Boston
Detwiler, S. R.	Columbia Univ.
Deuel, Harry J., Jr.	Univ. of S. Calif. Med.
Dick, George F.	Univ. of Chicago
Dickson, E. C.	Stanford Univ. Med.
Dieckmann, W. J.	Univ. of Chicago
Dienes, Louis	Massachusetts General Hospital, Boston
Dieuaide, Francis R.	Peiping Union Medical Coll.
Doan, Charles A.	Ohio State Univ.
Dochez, A. R.	Presbyterian Hospital, N. Y. City
Dock, William	Stanford Univ.
Doisy, Edward A.	St. Louis Univ.
Dolley, W. L., Jr.	Univ. of Buffalo
Dominguez, R.	St. Luke's Hospital, Cleveland
Donaldson, H. H.	Wistar Institute, Philadelphia
Donaldson, J. C.	Univ. of Pittsburgh
Dooley, M. S.	Syracuse Univ.
Doull, J. A.	Western Reserve Univ.
Downes, Helen R.	Memorial Hospital, N. Y. City
Drabkin, D. L.	Univ. of Pennsylvania
Dragstedt, Carl A.	Northwestern Univ.
Dragstedt, Lester R.	Univ. of Chicago
Draper, George W.	Columbia Univ.
Dresbach, M.	Albany Medical Coll.
Drury, D. R.	Univ. of S. Calif. Med.
Dubin, Harry E.	Metz Laboratories, N. Y. City
DuBois, E. F.	Cornell Univ. Medical Coll.
DuBois, F. S.	Univ. of Alabama
Duggar, B. M.	Univ. of Wisconsin
Dukes, H. H.	Cornell Univ.
Duran-Reynals, F.	Barcelona, Spain
Dunn, Max	Univ. of California, Los Angeles
Durrant, Edwin P.	Ohio State Univ.
Dusser de Barenne, J. G.	Yale Univ.
Dutcher, R. Adams	Pennsylvania State Coll.
Duval, C. W.	Tulane Univ.
Du Vigneaud, Vincent	Georgetown Univ. Med.
Dye, Joseph A.	Cornell Univ. Med.
Eberson, Frederick	San Francisco, Calif.
Ecker, E. E.	Western Reserve Univ.
Eckstein, Henry C.	Univ. of Michigan
Eddy, Walter H.	Columbia Univ.
Edmunds, C. W.	Univ. of Michigan
Edward, D. J.	Cornell Univ. Medical Coll.
Edwards, John G.	Univ. of Buffalo
Eggston, Andrew A.	N. Y. Manhattan Eye, Ear Hospital
Ellis, Max M.	Univ. of Missouri
Ellsworth, R. M.	Johns Hopkins Univ.
Elsberg, Charles A.	Neurological Institute, N. Y. City

Elser, W. J.	Cornell Univ. Medical Coll.
Emerson, G. A.	West Virginia Univ. Med.
Emery, F. E.	Univ. of Buffalo
Emge, L. A.	Stanford Univ.
Engle, E. T.	Columbia Univ.
Epstein, A. A.	Mt. Sinai Hospital, N. Y. City
Erlanger, Joseph	Washington Univ.
Ernstene, Arthur C.	Cleveland Clinic
Essex, Hiram E.	Mayo Clinic
Evans, Alice C.	National Inst. of Health, Washington
Evans, Herbert M.	Univ. of California
Everett, M. R.	Univ. of Oklahoma Med.
Ewing, James	Cornell Univ. Medical Coll.
Eyster, J. A. E.	Univ. of Wisconsin
Faber, Harold K.	Stanford Univ. Med.
Faehr, George	Univ. of Minnesota
Falk, I. S.	New Canaan, Conn.
Falk, K. George	N. Y. Univ. Medical Coll.
Falls, Frederick H.	Univ. of Illinois
Famulener, L. W.	St. Luke's Hospital, N. Y. City
Farmer, Chester	Northwestern Univ.
Faust, Ernest C.	Tulane Univ.
Feil, Harold	Western Reserve Univ.
Feng, T. P.	Peiping Union Med.
Fenn, Wallace O.	Univ. of Rochester Med.
Ferraro, Armando	N. Y. State Psychiatric Institute
Ferry, R. M.	Harvard Univ.
Field, John, II	Stanford Univ.
Figge, F. H. J.	Univ. of Maryland Med.
Fine, M. S.	Battle Creek, Mich.
Firor, W. M.	Johns Hopkins Univ.
Fischer, Albert	Kopenhagen, Denmark
Fischer, E.	Medical Coll., Va.
Fischer, Martin H.	Univ. of Cincinnati
Fishberg, Ella H.	Beth Israel Hospital, N. Y. City
Fitch, C. P.	Univ. of Minnesota
Fitz, Reginald	Peter Bent Brigham Hospital
Fitzgerald, J. G.	Univ. of Toronto
Fleisher, Moyer S.	St. Louis Univ.
Flinn, Frederick B.	Columbia Univ.
Florence, Laura	N. Y. Homeopathic Med.
Fluhmann, Charles F.	Stanford Univ. Med.
Forbes, Henry	Milton, Mass.
Forkner, Claude E.	Peiping Union Medical Coll.
Fortuyn, A. B. D.	Peiping Union Medical Coll.
Foster, Goodwin L.	Columbia Univ.
Fowler, Willis M.	State Univ. of Iowa
Fox, D. L.	Scripps Institution, LaJolla, Calif.
Frank, Robert T.	Mt. Sinai Hospital
Frazier, Chester N.	Peiping Union Medical Coll.

Freedlander, S. O.	Western Reserve Univ.
Freedman, Louis	Rensselaer, N. Y.
Freeman, R. G., Jr.	Columbia Univ.
Freund, Jules	Cornell Univ. Medical Coll.
Fridericia, L. S.	Univ. of Copenhagen, Denmark
Friedemann, T. E.	Univ. of Chicago
Friedman, M. H.	Univ. of Pennsylvania
Frobisher, Martin, Jr.	Baltimore, Md.
Fry, Henry J.	Cornell Medical Coll.
Fulmer, Ellis I.	Iowa State Coll.
Fulton, John F.	Yale Med.
Funk, Casimir	Paris, France
Furth, Jacob	Cornell Univ. Medical Coll.
Gaebler, O. H.	Henry Ford Hospital, Detroit
Gage, I. M.	Tulane Univ.
Gager, C. Stuart	Brooklyn Botanic Garden, N. Y.
Gallagher, T. F.	Univ. of Chicago
Gamble, James L.	Harvard Univ.
Garbat, Abraham L.	Lenox Hill Hospital, N. Y. City
Gardner, Leroy U.	Saranac Lab. Tuberculosis
Garrey, Walter E.	Vanderbilt Univ.
Gaskell, John F.	Cambridge, England
Gasser, Herbert S.	Rockefeller Institute
Gay, F. P.	Columbia Univ.
Geiling, E. M. K.	Univ. of Chicago
Geist, Samuel H.	Mount Sinai Hospital, N. Y. City
Gelfan, Samuel	Univ. of Illinois Medical Coll.
Gellhorn, Ernst	Univ. of Illinois Medical Coll.
Gengerelli, J. A.	Univ. of California, Los Angeles
Gerard, R. W.	Univ. of Chicago
Gerstenberger, H. J.	Western Reserve Univ.
Gesell, Robert A.	Univ. of Michigan
Gettler, A. O.	N. Y. Univ. Medical Coll.
Geyelin, H. R.	Columbia Univ.
Gibbs, O. S.	Univ. of Tenn.
Gibson, R. B.	Univ. of Iowa
Gies, William J.	Columbia Univ.
Gilchrist, Francis G.	Pomona Coll.
Gilligan, Dorothy R.	Beth Israel Hospital, Boston
Gilman, Alfred	Yale Univ.
Gilson, A. S., Jr.	Washington Univ. Med.
Githens, T. S.	Mulford Company, Philadelphia, Pa.
Givens, Maurice H.	Northwestern Yeast Co., Chicago
Glaser, Otto C.	Amherst Coll.
Glasser, Otto	Cleveland Clinic
Glick, D.	Mt. Zion Hospital, San Francisco
Goettsch, E.	Columbia Univ.
Gold, Harry	Cornell Univ. Med. Coll.
Goldberg, S. A.	Presbyterian Hospital, Newark, N. J.
Goldblatt, Harry	Western Reserve Univ.

Goldbloom, A. Allen	Beth Israel Hospital, N. Y. City
Goldforb, A. J.	Coll. of the City of New York
Goldring, W.	N. Y. Univ. Med.
Goldschmidt, Samuel	Univ. of Pennsylvania
Goodman, L.	Yale Univ.
Goodner, Kenneth	Rockefeller Institute
Gortner, R. A.	Univ. of Minnesota
Goss, C. M.	Columbia Univ.
Goss, Harold	Univ. of California
Gould, Harley N.	Tulane Univ.
Graeser, James B.	Univ. of California
Graham, Evarts A.	Washington Univ.
Graham, Helen T.	Washington Univ.
Graves, William W.	St. Louis Univ.
Gray, H.	Stanford Hospital
Gray, Samuel H.	Jewish Hospital, St. Louis, Mo.
Green, Robert G.	Univ. of Minnesota
Greenberg, David N.	Univ. of California
Greene, Carl H.	Brooklyn, N. Y.
Greene, James A.	State Univ. of Iowa
Greenwald, Isidor	N. Y. Univ. Medical Coll.
Greenwood, Alan	Univ. of Edinburgh
Gregg, D. E.	Western Reserve Univ.
Gregory, Louise H.	Barnard Coll., Columbia Univ.
Gregory, Paul W.	Univ. of California
Greisheimer, Esther M.	Women's Medical Coll., Philadelphia, Pa.
Griffith, Fred R., Jr.	Univ. of Buffalo
Griffith, Wendell H.	St. Louis Univ.
Gross, Erwin G.	State Univ. of Iowa
Gross, Louis	Mt. Sinai Hospital, N. Y. City
Gruber, Charles M.	Jefferson Medical Coll.
Guberlet, J. E.	Univ. of Washington
Guenther, A. E.	Univ. of Nebraska
Guerrant, N. B.	Pennsylvania State Coll.
Gunn, Francis D.	Northwestern Univ. Med.
Gurchot, Charles	San Francisco, Calif.
Gustavson, R. G.	Univ. of Denver
Gustus, E. L.	Upjohn Co., Michigan
Guthrie, C. C.	Univ. of Pittsburgh
Guy, Ruth A.	Peiping Union Medical Coll.
Guyer, Michael F.	Univ. of Wisconsin

Hagg, Harvey B.	Med. Coll. of Virginia
Hadley, Philip	Western Pennsylvania Hospital, Pittsburgh
Hafkesbring, H. Roberta	Woman's Med. Coll., Philadelphia
Hagan, William Arthur	Cornell Univ.
Hall, Ernest M.	Univ. of S. California Med.
Hall, V. E.	Stanford Univ.
Halsey, John	Tulane Univ.
Halsey, Robert H.	N. Y. Post-Graduate Med.

Hambourger, Walter E.	Yale Univ.
Hamburger, W. W.	Rush Medical Coll.
Hamilton, B. K.	Chicago, Illinois
Hammer, B. W.	Iowa State Coll.
Hanger, Franklin M.	Presbyterian Hospital, N. Y. City
Hanke, Martin E.	Univ. of Chicago
Hannon, R. R.	Peiping Union Medical Coll.
Hansmann, G. H.	Columbia Hospital, Milwaukee, Wis.
Hanzal, R. F.	Western Reserve Univ.
Hanzlik, P. J.	Stanford Univ. Med.
Harde, E.	Pasteur Inst., Paris
Harris, Isaac F.	Tuckahoe, N. Y.
Harris, William H.	Tulane Univ.
Harrison, R. G.	Yale Univ.
Harrop, George A., Jr.	Johns Hopkins Univ.
Harrow, Benjamin	Coll. of the City of New York
Hart, Edwin B.	Univ. of Wisconsin
Hart, George H.	Univ. Farm, Davis, Calif.
Hartman, F. A.	Ohio State Univ.
Hartman, F. W.	Ford Hospital, Detroit
Harvey, E. Newton	Princeton Univ.
Harvey, Samuel C.	Yale Univ.
Hastings, A. Baird	Harvard Univ.
Hastings, E. G.	Univ. of Wisconsin
Haterius, H. O.	New York Univ.
Hathaway, Edward S.	Tulane Univ.
Hawk, P. B.	New York City
Hawkins, James A.	U. S. Navy Yards, Washington, D. C.
Hayden, Charles E.	Cornell Univ.
Hayman, J. M., Jr.	Western Reserve Univ.
Haythorn, Samuel R.	Singer Research Lab., Pittsburgh, Pa.
Heft, Hattie L.	Columbia Univ.
Heidelberger, Michael	Presbyterian Hospital, N. Y. City
Heilbrunn, L. V.	Univ. of Pennsylvania
Heinbecker, Peter	Washington Univ.
Helff, O. M.	New York Univ.
Helmer, O. M.	Indiana Univ.
Helmholz, Henry R.	Mayo Clinic
Hendrix, B. M.	Univ. of Texas
Henrici, Arthur T.	Univ. of Minnesota
Henshaw, P. S.	Memorial Hospital, N. Y. City
Herrmann, G. R.	John Sealy Hospital, Galveston, Texas
Herrold, Russell D.	Univ. of Illinois Med.
Hertzman, A. B.	St. Louis Univ.
Hess, Walter C.	Georgetown Univ. Med.
Hewitt, E. A.	Iowa State Coll.
Heymans, J. C.	U. de Gand, Belgium
Hill, F. C.	Creighton Univ. Med.
Himwich, H. E.	Albany Medical Coll.
Hines, H. M.	State Univ. of Iowa

Hinman, Frank	Univ. of California Med.
Hinrichs, Marie Agnes	Univ. of Chicago
Hinsey, J. C.	Stanford Univ.
Hinshaw, H. C.	Mayo Clinic
Hirschfelder, Arthur	Univ. of Minnesota
Hisaw, F. L.	Harvard Univ.
Hitchcock, Fred A.	Ohio State Univ.
Hobmaier, Michael	Univ. of California
Hoeppli, R. J. C.	Peiping Union Medical Coll.
Hoffman, George L.	Allegheny County Hospital, Pittsburgh
Hoffman, W. S.	Chicago Med.
Hogan, Albert G.	Univ. of Missouri
Hollander, Franklin	Columbia Univ.
Holm, George E.	Dept. Agriculture, Washington, D. C.
Holman, Emil	Stanford Univ.
Holman, W. L.	Univ. of Toronto
Holmes, S. J.	Univ. of California
Holt, L. Emmett, Jr.	Johns Hopkins Univ.
Hooker, Davenport	Univ. of Pittsburgh
Hooker, Sanford B.	Boston Univ.
Hooper, Charles W.	Rensselaer, N. Y.
Hopkins, J. Gardner	Columbia Univ.
Hopkins, William	Univ. of California
Horsley, J. Shelton	St. Elizabeth's Hospital, Richmond, Va.
Hou, Hsiang-Chuan	Lester Inst. of Med. Research, Shanghai
Howe, Paul E.	Dept. Agriculture, Washington, D. C.
Howell, K. M.	Michael Reese Hospital, Chicago
Hu, C. H.	Peiping Union Med.
Hubbard, Roger S.	Buffalo General Hospital
Hudson, N. Paul	Ohio State Univ.
Hughes, Thomas P.	Rockefeller Institute
Humphrey, R. R.	Univ. of Buffalo
Hunscher, H. A.	Detroit, Mich.
Hunt, Reid	Harvard Univ.
Hunter, Warren C.	Univ. of Oregon Med.
Huntoon, F. M.	Woodbridge, Conn.
Hussey, Raymond	Yale Univ.
Irwin, Marion	Rockefeller Inst.
Irwin, M. R.	Univ. of Wisconsin
Isaacs, Raphael	Simpson Memorial Institute, Ann Arbor
Ivy, Andrew C.	Northwestern Univ.
Jackson, C. M.	Univ. of Minnesota
Jackson, D. E.	Univ. of Cincinnati
Jackson, Henry, Jr.	Boston City Hospital
Jackson, Richard W.	Cornell Univ. Medical Coll.
Jacobs, Henry R.	Univ. of Chicago
Jacobs, M. H.	Univ. of Pennsylvania
Jacobs, Walter A.	Rockefeller Institute, N. Y. City

Jacobsen, Victor C.	Troy, N. Y.
Jaffe, Henry L.	Hospital for Joint Diseases, N. Y. City
Jaffe, Richard H.	Univ. of Illinois
Jeans, Philip C.	Univ. of Iowa
Jennings, H. S.	Johns Hopkins Univ.
Jensen, Hans F.	Johns Hopkins Univ.
Jobling, J. W.	Columbia Univ.
Johlin, J. M.	Vanderbilt Univ. Med.
Johns, Foster M.	Tulane Univ.
Johnson, C. A.	Univ. of Chicago
Johnson, C. C.	Univ. of Utah
Johnson, T. B.	Yale Univ.
Jolliffe, Norman H.	N. Y. Univ. Medical Coll.
Jonas, Leon	Univ. of Pennsylvania
Jones, L. R.	St. Louis Univ.
Jordan, Edwin	Univ. of Chicago
Jordan, H. E.	Univ. of Virginia
Jorstad, L. H.	Barnard Skin and Cancer Hospital, St. Louis, Mo.
Joslin, E. P.	Boston, Mass.
Juhn, Mary	Univ. of Chicago
Jukes, T. H.	Univ. of California
Julianelle, Louis A.	Washington Univ.
Jung, F. T.	Northwestern Univ. Med.
Jungeblut, Claus	Coll. of Physicians and Surgeons, N. Y.

Kahn, Morton C.	Cornell Medical Coll., N. Y. City
Kahn, R. L.	Univ. Hospital, Ann Arbor, Mich.
Kamm, Oliver	Parke, Davis & Co., Detroit
Karsner, H. T.	Western Reserve Univ.
Kast, Ludwig	N. Y. Post-Graduate Med.
Katayama, I.	St. Luke's International Hospital, Tokyo
Katz, L. N.	Michael Reese Hospital, Chicago
Katzenelbogen, S.	Johns Hopkins Univ.
Katzman, Philip A.	St. Louis Univ.
Keefer, Chester S.	Boston City Hospital
Keeton, Robert W.	Univ. of Illinois
Keitt, G. W.	Univ. of Wisconsin
Keller, Allen D.	Univ. of Alabama Med.
Kellogg, W. H.	Univ. of California
Kemp, Hardy A.	Baylor Univ. Medical Coll.
Kendall, Arthur I.	Northwestern Univ. Med.
Kendall, E. C.	Mayo Clinic, Minn.
Kerr, William J.	Univ. of California Med.
Kessel, John F.	Univ. of S. California
Kesten, H. D.	Coll. of Physicians and Surgeons
Key, John A.	Washington Univ.
Killian, J. A.	N. Y. Post-Graduate Med.
Kindred, J. E.	Univ. Va. Med.
King, Helen D.	Wistar Institute, Philadelphia
Kinsella, Ralph A.	St. Louis Univ.

Kirkbride, Mary B.	State Dept. of Health, Albany, N. Y.
Kleiber, Max	Univ. of California, Davis
Kleiner, I. S.	N. Y. Homeopathic Med.
Kleitman, Nathaniel	Univ. of Chicago
Kligler, I. J.	Hebrew Univ., Palestine
Kline, B. S.	Mt. Sinai Hospital, Cleveland, Ohio
Klotz, Oskar	Univ. of Toronto
Klüver, H.	Univ. of Chicago
Knoefel, P. K.	Vanderbilt Univ.
Knowlton, Frank P.	Syracuse Univ.
Knudson, Arthur	Albany Medical Coll.
Kober, Philip A.	G. D. Searle and Co., Chicago
Koch, Fred C.	Univ. of Chicago
Koehler, Alfred E.	Santa Barbara Hospital, Calif.
Kofoid, Charles A.	Univ. of California
Kohn, Jerome L.	Mt. Sinai Hospital, N. Y. City
Kolmer, John A.	Univ. of Pennsylvania
Kopeloff, Nicholas	Psychiatric Institute, N. Y. City
Koppányi, Theodore	Georgetown Univ. Med.
Korns, Horace M.	State Univ. of Iowa
Koser, Stewart	Univ. of Chicago
Kramer, Benjamin	Brooklyn Jewish Hospital
Kramer, S. D.	Long Island Coll. of Medicine
Krantz, J. C., Jr.	Univ. of Maryland Med.
Krasnow, Frances	Guggenheim Dental Clinic, N. Y. City
Kriss, M.	Pa. State Coll.
Krizenecky, J.	Brno, Czechoslovakia
Kronfeld, P. C.	Peiping Union Med.
Krueger, A. P.	Univ. of California
Krumbhaar, E. B.	Univ. of Pennsylvania
Kruse, Theophile K.	Univ. of Pittsburgh
Kugelmass, I. Newton	N. Y. City
Kunde, Margaret M.	Chicago Med.
Kunitz, Moses	Rockefeller Institute, Princeton, N. J.
Kuntz, Albert	St. Louis Univ.
Kurotchkin, T. J.	Peiping Union Medical Coll.
Kurzrok, Raphael	Coll. of Physicians and Surgeons

Ladd, William S.	Presbyterian Hospital, N. Y. City
Lambert, R. A.	Rockefeller Foundation
Lamson, Paul D.	Vanderbilt Univ.
Lamson, Robert W.	Los Angeles, Calif.
Lancefield, D. E.	Columbia Univ.
Landis, Carney	Psychiatric Institute, N. Y. City
Landsteiner, Karl	Rockefeller Institute, N. Y. City
Langstroth, Lovell	San Francisco, California
Larkum, N. W.	Michigan Department of Health
Larsell, Olaf	Univ. of Oregon
Larson, Edward	Temple University
Larson, W. P.	Univ. of Minnesota

Lathrop, Carl O.	Univ. of Buffalo
Laughlin, H. H.	Exp. Evolution, Cold Spring Harbor, N. Y.
Laurens, Henry	Tulane Univ.
Leake, C. D.	Univ. of California Med.
Leake, J. P.	Hygienic Laboratory, Washington, D. C.
Lee, Ferdinand C.	Johns Hopkins Med.
Lee, Frederic S.	Columbia Univ.
Lee, Milton O.	Harvard Univ.
Leiter, Louis	Univ. of Chicago
Leonard, S. L.	Union Coll.
Leonard, Veader	Johns Hopkins Univ
Levene, P. A.	Rockefeller Institute, N. Y. City
Levin, Isaac	New York City
Levine, Max	Iowa State Coll.
Levine, Michael	Montefiore Hospital, N. Y. City
Levine, Philip	Univ. of Wisconsin
Levine, Samuel Z.	Cornell Univ. Medical Coll.
Levine, Victor E.	Creighton Univ.
Levinson, Samuel A.	Univ. of Illinois
Levy, Milton	N. Y. Univ. Medical Coll.
Levy, Robert L.	Presbyterian Hospital, N. Y. City
Lewis, Howard B.	Univ. of Michigan
Lewis, Robert C.	Univ. of Colorado
Liddell, H. S.	Cornell Univ.
Lieb, C. C.	Columbia Univ.
Lillie, Frank R.	Univ. of Chicago
Lillie, Ralph S.	Univ. of Chicago
Lim, C. E.	Peiping Union Medical Coll., China
Lim, Robert K.	Peiping Union Medical Coll., China
Lindegren, C. C.	Univ. S. Calif.
Ling, Schmorl M.	Peiping Union Medical Coll., China
Linton, Richard W.	Calcutta, India
Lipman, Charles B.	Univ. of California
Little, C. C.	Bar Harbor, Maine
Liu, Shih-hao	Peiping Union Medical Coll., China
Locke, A. P.	W. Pennsylvania Hospital, Pittsburgh
Loeb, Leo	Washington Univ.
Loeb, Robert F.	Presbyterian Hospital, N. Y. City
Loebel, Robert O.	N. Y. City
Loehwing, W. F.	Univ. of Iowa
London, E. S.	Leningrad, U. S. S. R.
Long, C. H. N.	Univ. of Pennsylvania
Long, E. R.	Henry Phipps Institute, Philadelphia
Long, Perrin H.	Johns Hopkins Hospital
Longcope, W. T.	Johns Hopkins Univ.
Looney, J. M.	Worcester State Hospital, Mass.
Lorenz, William F.	Univ. of Wisconsin
Lucas, William P.	Univ. of Calif.
Lucia, S. P.	Univ. of Calif.
Luck, J. Murray	Stanford Univ.

Lucké, Baldwin	Univ. of Pennsylvania
Luckhardt, A. B.	Univ. of Chicago
Lueth, H. C.	Northwestern Univ.
Lund, E. J.	Univ. of Texas
Lurie, Max B.	Henry Phipps Institute, Philadelphia
Luyet, B. J.	St. Louis Univ.
Lyman, J. F.	Ohio State Univ.
Lynch, Clara J.	Rockefeller Institute, N. Y. City
Lyon, E. P.	Univ. of Minnesota
Lyons, W. R.	Univ. of Calif.

McCann, William S.	Univ. of Rochester
McClendon, J. Francis	Univ. of Minnesota
McClintock, John T.	Univ. of Iowa
McCollum, E. V.	Johns Hopkins Univ.
McCordock, H. A.	Washington Univ.
McCoy, Oliver R.	Univ. of Rochester
McCullagh, D. R.	Cleveland Clinic Foundation
McCutcheon, Morton	Univ. of Pennsylvania
McEachern, Donald	Univ. of Pittsburgh
McEllroy, W. S.	Univ. of Pittsburgh
McGinty, Daniel A.	Parke, Davis Co.
McIntosh, Rustin	Babies Hospital, N. Y. City
McIver, M. A.	Bassett Hospital, Cooperstown, N. Y.
McJunkin, Frank A.	Loyola Univ.
McKibben, Paul S.	Univ. of S. California
McKinley, E. B.	George Washington Univ
McKinley, J. C.	Univ. of Minnesota
McLean, Franklin C.	Univ. of Chicago
McMaster, Philip D.	Rockefeller Institute, N. Y. City
McMeans, J. W.	Pittsburgh Hospital
McQuarrie, Irvine	Univ. of Minnesota
Ma, W. C.	Peiping Union Medical Coll., China
MacDowell, E. Carlton	Exp. Evolution, Cold Spring Harbor, N. Y.
Macht, D. I.	Baltimore, Md.
MacKay, E. M.	Scripps Metabolic Clinic, San Diego, Calif.
Mackenzie, George M.	Bassett Hosp., Cooperstown, N. Y.
MacLeod, Grace	Columbia Univ.
MacNeal, Ward J.	N. Y. Post-Graduate Med.
MacNider, William de B.	Univ. of North Carolina
Macy, I. G.	Detroit, Mich.
Magath, T. B.	Mayo Clinic
Malamud, William	Iowa State Psychopathic Hospital
Mallman, W. Leroy	Michigan State Coll.
Mallory, Frank	Boston City Hospital
Mallaner, E. J.	N. Y. State Dept. of Health
Maltaner, Frank	State Dept. Health, Albany, N. Y.
Mandel, Arthur R.	N. Y. Univ. Medical Coll.
Mann, Frank C.	Mayo Clinic
Mann, Hubert	U. S. Veterans' Hospital, N. Y. City

Manville, Ira Albert	Univ. of Oregon
Manwaring, W. H.	Stanford Univ. Med.
Marine, David	Montefiore Hospital, N. Y. City
Markowitz, Jacob	Toronto, Canada
Marmorsten, J.	Cornell Medical Coll.
Marriott, McKim	St. Louis Children's Hospital, St. Louis
Marsh, R. P.	Gettysburg Coll.
Marshall, E. K., Jr.	Johns Hopkins Univ.
Marshall, Max S.	Univ. of California
Martin, Lay	Johns Hopkins Univ.
Mason, K. E.	Vanderbilt Univ.
Master, Arthur M.	N. Y. City
Matas, Rudolph	New Orleans, La.
Mattill, Henry A.	State Univ. of Iowa
Maughan, G. H.	Cornell Univ.
Mayerson, H. S.	Tulane Univ.
Maynard, L. A.	Cornell Univ.
Meakins, Jonathan	McGill Univ.
Medes, Grace	Lankenau Research Inst., Philadelphia
Medlar, E. M.	Mt. McGregor, N. Y.
Meek, Walter J.	Univ. of Wisconsin
Megrail, Emerson	Western Reserve Univ.
Meigs, E. B.	Dairy Division Exp. Station, Beltsville, Md.
Meleney, F. L.	Presbyterian Hospital, N. Y. City
Melhus, I. E.	Iowa State Coll.
Mellon, Ralph R.	Western Pennsylvania Hospital, Pittsburgh
Menkin, Valy	Harvard Med.
Menten, Maude L.	Univ. of Pittsburgh
Menville, Leon J.	Tulane Univ.
Mettier, Stacy R.	Univ. of California Med.
Meyer, Adolph L.	Johns Hopkins Univ.
Meyer, A. E.	N. Y. City
Meyer, A. W.	Stanford Univ. Med.
Meyer, Karl	Columbia Univ.
Meyer, K. F.	Univ. of California
Meyer, O. O.	Univ. of Wisconsin
Meyer, R. K.	Univ. of Wisconsin
Michaelis, Leonor	Rockefeller Institute
Michels, N. A.	Jefferson Med.
Middleton, W. S.	Univ. of Wisconsin
Miles, W. R.	Yale University
Milhorat, A. T.	Cornell Medical Coll.
Miller, C. Phillip, Jr.	Univ. of Chicago
Miller, E. G., Jr.	Columbia Univ.
Miller, Franklin R.	Western Reserve Univ.
Miller, G. H.	American Univ. of Beirut, Syria
Miller, Joseph L.	St. Luke's Hospital, Chicago
Millet, John A. P.	New York City
Mills, Clarence A.	Cincinnati General Hospital
Minot, George R.	Boston City Hospital

Mirsky, Alfred E.	Rockefeller Institute
Mitchell, Helen S.	Massachusetts State Coll.
Mitchell, O. W. H.	Syracuse Univ.
Moberg, Erik G.	Scripps Institute, La Jolla, California
Moon, V. H.	Jefferson Medical Coll.
Moore, A. R.	Univ. of Oregon
Moore, Carl R.	Univ. of Chicago
Moore, M. M.	Univ. of Oregon
Moore, R. A.	Cornell Medical Coll.
Moore, R. M.	Univ. of Texas Med., Galveston
Morgan, A. F.	Univ. of California
Morgan, T. H.	California Institute of Technology
Morgulis, Sergius	Univ. of Nebraska Coll. of Medicine
Moritz, A. R.	Western Reserve Univ.
Morrell, J. A.	E. R. Squibb and Sons
Morse, Arthur	New Haven Hospital
Morton, John J.	Univ. of Rochester Med.
Mosenthal, H. O.	N. Y. Post-Graduate Med.
Mu, J. W.	Peiping Union Med.
Muckenfuss, R. S.	N. Y. City Bd. of Health
Mudd, Stuart	Univ. of Pennsylvania
Mudge, C. S.	Univ. of California
Muehlberger, C. W.	Northwestern Univ.
Mueller, J. Howard	Harvard Univ.
Mulinos, M. G.	Columbia Univ.
Mull, J. W.	Western Reserve Univ.
Muller, G. L.	Boston City Hospital
Muller, H. J.	Univ. of Texas
Muntwyler, Edward	Western Reserve Univ.
Murlin, John R.	Univ. of Rochester
Murphy, D. P.	Univ. of Pennsylvania
Murphy, J. B.	Rockefeller Institute
Murray, Thomas J.	Rutgers Univ.
Musser, John H.	Tulane Univ.
Myers, Chester N.	Columbia Univ.
Myers, Harold B.	Univ. of Oregon
Myers, Victor C.	Western Reserve Univ.

Nathanson, Morris H.	Univ. of Minnesota
Necheles, H.	Univ. of Chicago
Nedzel, A. J.	Univ. of Illinois Med.
Nelson, Erwin E.	U. S. Dept. of Agri., Washington, D. C.
Nelson, Thurlow C.	Rutgers Univ.
Nelson, Victor E.	Iowa State Coll.
Nelson, W. O.	Yale Univ.
Neuwirth, Isaac	New York Univ.
Newburgh, Louis H.	Univ. of Michigan
Ni, Tsang-gi	Peiping Union Medical Coll.
Nice, L. B.	Ohio State Univ.
Nicholas, J. S.	Yale Univ.

Nicholls, Edith E.	Cornell Univ. Medical Coll.
Nichols, M. S.	Univ. of Wisconsin
Nicholson, Hayden C.	Univ. of Michigan
Nielsen, Carl	Abbott Laboratories, Chicago
Noble, G. Kingsley, Jr.	Amer. Museum Natural History, N. Y. City
Norris, Earl R.	Univ. of Washington
Norris, L. C.	Cornell Univ.
Northrop, John H.	Rockefeller Institute, Princeton, N. J.
Norton, J. F.	Upjohn Co., Kalamazoo, Mich.
Novy, Frederick G.	Univ. of Michigan
Nungester, W. J.	Northwestern Univ. Med.
Nye, R. N.	Boston City Hospital

Obreshkove, Vasil	St. Stephen's Coll.
Ochsner, E. W. A.	Tulane Univ.
Oertel, Horst	McGill Univ.
Okey, Ruth E.	Univ. of California
O'Leary, J. L.	Washington Univ.
Olitsky, Peter K.	Rockefeller Institute, N. Y. City
Oliver, Jean	Long Island Coll., Brooklyn
Oliver, W. W.	Long Island Coll. of Medicine
Olmstead, Miriam P.	Presbyterian Hospital, N. Y. City
Olmstead, W. H.	Washington Univ.
Olmsted, J. M. D.	Univ. of California
Opie, Eugene L.	Cornell Univ. Medical Coll.
Oppenheimer, B. S.	Columbia Univ.
Orent, Elsa	Johns Hopkins Univ.
Ornstein, George G.	Columbia Univ.
Orten, James M.	Yale Univ.
Osgood E. E.	Univ. of Oregon Med.
Oslund, R. M.	Los Angeles, Calif.
Osterhout, W. J. V.	Rockefeller Institute
Ottenberg, R.	Mt. Sinai Hospital, N. Y. City
Otto, H. L.	N. Y. Univ. Medical Coll.
Oughterson, A. W.	Yale Univ.

Pack, George T.	Memorial Hospital, N. Y. City
Page, Irvine H.	Rockefeller Institute, N. Y. City
Pak, Chubyung	Lester Institute Med. Research, Shanghai
Palmer, Leroy S.	Univ. of Minnesota
Palmer, W. L.	Univ. of Chicago
Palmer, W. W.	Presbyterian Hospital, N. Y. City
Papanicolaou, George N.	Cornell Univ. Medical Coll.
Pappenheimer, A. M.	Columbia Univ
Park, E. A.	Rodgers Forge, Maryland
Park, William H.	Department of Health, N. Y. City
Parker, George H.	Harvard Univ.
Parr, L. W.	George Washington Univ.
Parsons, Helen T.	Univ. of Wisconsin
Paul, William D.	State Univ. of Iowa

Pearce, Louise	Rockefeller Institute
Pearl, Raymond	Johns Hopkins Univ.
Pearse, Herman E.	Univ. of Rochester
Pease, Marshall C.	N. Y. Post-Graduate Med.
Pellini, Emil J.	N. Y. Univ. Medical Coll.
Pemberton, Ralph	Memorial Hospital, Abington, Pa.
Pencharz, R. I.	Univ. of California
Penfield, Wilder G.	McGill Univ.
Pepper, O. H. Perry	Univ. of Pennsylvania
Perla, David	Montefiore Hospital, N. Y. City
Perlzweig, William A.	Duke Univ.
Permar, Howard H.	Mercy Hospital, Pittsburgh
Peskind, Samuel	Cleveland, Ohio
Peters, John P.	Yale Univ.
Petersen, W. E.	Univ. of Minnesota
Petroff, S. A.	Trudeau, N. Y.
Pfeiffer, J. A. F.	Johns Hopkins Univ.
Pfiffner, J. J.	Coll. Physicians and Surgeons, N. Y. City
Phemister, D. B.	Univ. of Chicago
Pierce, Harold B.	Univ. of Rochester
Pilot, I.	Univ. of Illinois Med.
Pinkerton, Henry	Harvard Med.
Pinner, Max	Desert Sanitarium, Tucson, Arizona
Plant, O. H.	Univ. of Iowa
Plass, E. D.	Univ. of Iowa
Plotz, Harry	Pasteur Institute, Paris
Pohlman, Augustus G.	Creighton Univ.
Pollock, H.	Cornell Univ. Med.
Pollock, L. F.	Northwestern Univ.
Ponder, Eric	Cold Spring Harbor, N. Y.
Powell, H. M.	Eli Lilly and Co.
Powers, Grover F.	Yale Univ.
Pratt, Frederick H.	Boston Univ.
Pratt, Joseph H.	Harvard Univ.
Prewitt, Proviso	N. Y. Univ. Medical Coll.
Pribram, Ernest	Chicago, Ill.
Prince, A. L.	Hartford Hospital, Conn.
Proescher, F.	San José, Calif.
Puestow, C. B.	Univ. of Illinois Med.

Quick, Armand J.	Milwaukee, Wis.
Quigley, J. P.	Western Reserve Med.
Quinby, W. C.	Peter Bent Brigham Hospital
Quinn, Edmond John	Rockville Center, N. Y.

Ragins, Ida Kraus	Univ. of Chicago
Rahn, O.	Cornell Univ.
Raiziss, George W.	Inst. Cutaneous Medicine, Philadelphia
Rake, Geoffrey W.	Rockefeller Institute
Ralli, Elaine P.	N. Y. Univ. Medical Coll.

Ranson, S. W.	Northwestern Univ.
Rapport, David	Tufts Coll. Med.
Rasmussen, A. T.	Univ. of Minnesota
Ratner, Bret	N. Y. Univ. Medical Coll.
Ravdin, I. S.	Univ. of Pennsylvania
Ray, G. B.	Long Island Coll. of Medicine
Ray, Henry M.	S. Side Hospital, Pittsburgh
Read, Bernard E.	Lester Institute, Shanghai, China
Read, J. Marion	Stanford Univ. Med.
Reed, Carlos I.	Univ. of Illinois Med.
Reed, H. S.	Univ. of California
Reichert, F. L.	Stanford Univ.
Reichle, H. S.	Western Reserve Univ.
Reimann, Hobart A.	Univ. of Minnesota
Reimann, Stanley P.	Univ. of Pennsylvania
Reiner, L.	Burroughs Wellcome and Co.
Rettger, L. F.	Yale Univ.
Reynolds, Chapman	New Orleans
Reynolds, S. R.	Long Island Coll.
Reznikoff, Paul	Cornell Univ. Medical Coll.
Rhoads, Cornelius P.	Rockefeller Institute
Richards, Alfred N.	Univ. of Pennsylvania
Richards, Leah B.	Memorial Hospital, N. Y. City
Richardson, Henry B.	Cornell Univ. Medical Coll.
Richardson, R.	Univ. of Pennsylvania
Richey, DeWayne G.	Univ. of Pittsburgh
Richter Maurice	Columbia Univ.
Riddle, Oscar	Exp. Evolution, Cold Spring Harbor, N. Y.
Riebeth, A. B.	Minneapolis
Ringer, Michael	Montefiore Hospital, N. Y. City
Rivers, Thomas M.	Rockefeller Institute
Robb, Jane Sands	Syracuse Univ.
Roberts, Elmer	Univ. of Illinois
Robertson, Oswald H.	Univ. of Chicago
Robinson, Charles S.	Vanderbilt Univ.
Robinson, Elliott	Antitoxin Laboratory, Boston
Robinson, G. Canby	Cornell Univ. Medical Coll.
Roe, Joseph H.	George Washington Univ.
Rogers, Fred T.	Baylor Univ.
Rogoff, J. M.	Univ. of Chicago
Rohdenburg, George L.	Lenox Hill Hospital, N. Y. City
Romanoff, Alexis L.	Cornell Univ.
Ronzoni, Ethel	Washington Univ.
Roome, N. W.	Univ. of Chicago
Root, W. S.	Univ. of Maryland
Rosahn, Paul D.	Chicago, Ill.
Rose, Anton R.	Prudential Life Insurance Co., Newark, N. J.
Rose, Mary Swartz	Columbia Univ.
Rose, William C.	Univ. of Illinois
Rosenow, E. C.	Mayo Foundation

Rosenthal, Lazar	Israel-Zion Hospital, Brooklyn, N. Y.
Ross, Victor	Department of Health, N. Y. City
Roth, George B.	George Washington Univ.
Rous, Peyton	Rockefeller Institute
Rowntree, L. G.	Philadelphia Institute for Medical Research
Russell, W. C.	N. J. Agr. Exp. Station
Sabin, Florence R.	Rockefeller Institute
Sahyun, Melville	Detroit, Mich.
Salant, William	N. Y. City
Salle, A. J.	Univ. of California
Salvesen, Harold A.	Rikshospitalet, Oslo, Norway
Samuels, L. T.	Univ. of S. California, Los Angeles
Sandberg, M.	Montefiore Hospital, N. Y. City
Sanford, A. H.	Mayo Clinic
Sansum, W. D.	Santa Barbara Cottage Hospital, Calif.
Saslow, George	N. Y. Univ.
Sawyer, W. A.	Rockefeller Foundation
Scammon, R. E.	Univ. of Minnesota
Scheff, George	Hygienic Inst., Pécs, Hungary
Schick, Béla	Mt. Sinai Hospital, N. Y. City
Schlesinger, M. J.	Beth Israel Hospital, Boston
Schloss, Oscar M.	Cornell Univ. Medical Coll.
Schlutz, F. W.	Univ. of Chicago
Schmidt, Carl F.	Univ. of Pennsylvania
Schmidt, Carl L. A.	Univ. of California
Schmitt, Francis O.	Washington Univ.
Schneider, Edward C.	Wesleyan Univ.
Schultz, E. W.	Stanford Univ.
Schultz, O. T.	St. Francis Hospital, Evanston, Ill.
Schultz, W. H.	Univ. of Maryland
Schwartz, Benjamin	U. S. Dept. of Agriculture
Schwind, J. L.	Albany Med.
Scott, E. L.	Columbia Univ.
Scott, F. H.	Univ. of Minnesota
Scott, Gordon H.	Washington Univ.
Scott, J. M.	Univ. of Rochester
Scott, Leonard C.	Tulane Univ.
Scott, R. W.	Western Reserve Univ.
Sears, H. J.	Univ. of Oregon
Seecof, David P.	Jewish Gen. Hospital, Montreal
Seegal, Beatrice C.	Coll. of Physicians and Surgeons, N. Y. City
Seevers, M. H.	Univ. of Wisconsin
Seibert, Florence B.	Henry Phipps Institute, Philadelphia
Severinghaus, A. E.	Coll. of Physicians and Surgeons, N. Y. City
Sevringhaus, Elmer L.	Univ. of Wisconsin
Shaffer, Philip A.	Washington Univ.
Shaklee, A. O.	St. Louis Univ.
Shannon, J. A.	N. Y. Univ. Med.
Sharlit, Herman	Roosevelt Hospital, N. Y. City

Sharp, P. F.	Cornell Univ.
Shear, M. J.	Harvard Med.
Sheard, Charles	Mayo Clinic
Sheinin, J. J.	Chicago Med.
Shelling, D. H.	Jewish Hospital, N. Y. City
Sherman, H. C.	Columbia Univ.
Sherman, Hartley E.	Bureau of Science, Manila, P. I.
Sherman, James M.	Cornell Univ.
Sherwin, Carl P.	Fordham Univ.
Shibley, Gerald S.	Lakeside Hospital, Cleveland
Shilling, C. W.	Navy Yard, Washington, D. C.
Shiple, George J.	Univ. of Detroit
Shohl, Alfred T.	Boston, Mass.
Shope, R. E.	Rockefeller Inst., Princeton, N. J.
Shorr, E.	Coll. of Physicians and Surgeons, N. Y. City
Shwartzman, Gregory	Mt. Sinai Hospital, N. Y. City
Sia, Richard H. P.	Peiping Union Medical Coll.
Sickles, Grace M.	N. Y. State Dept. of Health
Silverman, D. N.	Tulane Univ.
Simmons, Major James S.	Army Med., Washington, D. C.
Simonds, J. P.	Northwestern Univ.
Sinclair, R. G.	Univ. of Rochester
Sittenfield, M. J.	Columbia University
Smetana, Hans	Columbia Univ.
Smith, Arthur H.	Yale Univ.
Smith, Carl H.	Cornell Medical Coll.
Smith, Clarence	Bergenfield, N. J.
Smith, Erma A.	Iowa State Coll.
Smith, Fred M.	Univ. of Iowa
Smith, George V.	Hospital for Women, Brookline, Mass.
Smith, Harry P.	State Univ. of Iowa
Smith, Homer W.	N. Y. Univ. Medical Coll.
Smith, Lawrence W.	Temple Univ., Philadelphia
Smith, Millard	Boston, Mass.
Smith, Philip E.	Columbia Univ.
Smithburn, K. C.	Rockefeller Institute
Smyly, H. Jocelyn	Shantung Christian Univ., China
Snyder, Franklin F.	Johns Hopkins Univ.
Sobotka, H. H.	Mt. Sinai Hospital, N. Y. City
Sollmann, Torald	Western Reserve Univ.
Sommer, Hermann	Univ. of California
Somogyi, Michael	Jewish Hospital, St. Louis, Mo.
Soskin, Samuel	Michael Reese Hospital, Chicago
Soule, M. H.	Univ. of Michigan
Sperry, Warren	Babies Hospital, N. Y. City
Spiegel, Ernst	Temple Univ.
Spiegel-Adolf, Mona	Temple Univ.
Spies, T. D.	Western Reserve Univ.
Sponsler, O. L.	Univ. of California, Los Angeles
Sprunt, D. H.	Duke Hospital, No. Carolina

Stainsby, W. J.	Cornell Univ. Medical Coll.
Stark, Clifford N.	Cornell Univ.
Stark, Mary B.	Homeopathic Medical Coll., N. Y. City
Starkey, R. L.	Agri. Exp. Station, New Brunswick, N. J.
Starr, M. P.	Northwestern Univ. Med.
Steinberg, Bernhard	Toledo Hospital, Toledo, Ohio
Steindler, A.	State Univ. of Iowa
Stekol, J. A.	Fordham Univ.
Stenstrom, Wilhelm	Univ. of Minnesota
Stewart, Harold J.	New York Hospital
Stiles, Percy G.	Harvard Univ.
Still, Eugene U.	Univ. of Chicago
Stillman, Ralph G.	Cornell Univ.
St. John, J. H.	Army Med.
Stockard, Charles R.	Cornell Univ. Med.
Stockton, A. B.	Stanford Univ. Med.
Stone, Calvin P.	Stanford Univ.
Stone, Leon S.	Yale Univ.
Storey, Thomas A.	Stanford Univ.
Stovall, W. D.	Univ. of Wisconsin
Strong, Richard P.	Harvard Univ.
Strouse, Solomon	Northwestern Univ.
Stuart, C. A.	Brown Univ.
Stucky, C. J.	Georgetown Univ.
Stunkard, Horace W.	New York Univ.
Sturgis, C. C.	Simpson Memorial Inst., Ann Arbor, Mich.
Sugiura, Kanematsu	Memorial Hospital, N. Y. City
Sullivan, M. X.	Georgetown Univ.
Sullivan, Walter E.	Univ. of Wisconsin
Sumner, F. B.	Scripps Institution, La Jolla, Calif.
Sumner, James B.	Cornell Univ.
Sundstroem, Edward S.	Univ. of California
Sure, Barnett	Univ. of Arkansas
Swanson, W. W.	Univ. of Chicago
Sweany, Henry C.	Univ. of Chicago
Sweet, J. Edwin	Cornell Univ. Medical Coll.
Swett, Francis H.	Duke Univ.
Swift, H. F.	Rockefeller Institute
Swindle, P. F.	Marquette Univ. Med
Swingle, W. W.	Princeton Univ.

Tainter, Maurice L.	Stanford Univ.
Tanner, Fred W.	Univ. of Illinois
Tashiro, Shiro	Univ. of Cincinnati
Tatum, A. L.	Univ. of Wisconsin
Tauber, Henry	N. Y. Homeopathic Medical Coll.
Ten-Broeck, Carl	Rockefeller Inst., Princeton, N. J.
Thalhimer, William	N. Y. City
Thayer, S. A.	St. Louis Univ.
Thienes, Clinton H.	Univ. of S. California

Thomas, Arthur W.	Columbia Univ.
Thomas, J. E.	Jefferson Medical Coll.
Thomas, Karl	Physiologisch-Chemisches Institut, Leipzig
Thomas, Walter S.	Clifton Springs, N. Y.
Thompson, Willard O.	Rush Medical Coll.
Thoraldsen, C. E.	N. Y. Homeopathic Med.
Thro, William C.	Cornell Univ. Medical Coll.
Tisdall, Frederick F.	Univ. of Toronto
Todd, T. Wingate	Western Reserve Univ.
Tolstoi, Edward	N. Y. City
Toomey, J. A.	Western Reserve Univ.
Torrey, Harry B.	Stanford Univ.
Torrey, J. C.	Cornell Univ. Medical Coll.
Trattner, H. R.	Western Reserve Univ.
Travis, L. E.	State Univ. of Iowa
Tripoli, C. J.	La. State Univ.
Tsen, Edgar T. H.	Epidemic Prevention Bureau, Peiping
Tunnicliff, Ruth	McCormick Institute, Chicago
Turner, Charles W.	Univ. of Missouri
Turner, Kenneth B.	Presbyterian Hospital, N. Y. City
Turner, Robert G.	Detroit Coll. of Medicine
Turner, Roy H.	Tulane Univ.
Tuttle, W. W.	State Univ. of Iowa
Tweedy, W. R.	Loyola Univ. Med.

Uhlenhuth, Edward	Univ. of Maryland
Uyei, N.	Univ. of Calif.

Van Dyke, H. B.	Peiping Union Medical Coll.
Van Liere, E. J.	Univ. of W. Virginia
Van Slyke, Donald D.	Rockefeller Institute
Vars, Harry M.	Univ. of Pennsylvania
Vaughan, Stuart L.	Buffalo General Hospital
Vaughn, T. Wayland	Scripps Institution, California
Vickery, H. B.	Conn. Agr. Exp. Station
Victor, Joseph	Coll. of Physicians and Surgeons
Visscher, J. Paul	Western Reserve Univ.
Visscher, Maurice B.	Univ. of Illinois Med.
Voegtlin, Carl	Nat'l Institute of Health, Washington, D. C.
Vogel, Karl M.	St. Luke's Hospital, N. Y. City
Von Haam, Hans E.	La. State Univ. Medical Center
Von Oettingen, W. F.	Wilmington, Del.

Wadsworth, Augustus B.	N. Y. State Dept. of Health
Wakerlin, G. E.	Univ. of Louisville
Waksman, S. A.	N. J. State Agr. Exp. Station
Walker, A. W.	Northwestern Univ.
Walker, E. L.	Univ. of California
Wallace, George B.	N. Y. Univ. Medical Coll.
Wallin, Ivan E.	Univ. of Colorado

Walton, Robert P.	Tulane Univ.
Walzer, Matthew	Brooklyn Jewish Hospital
Wang, Chi Che	Children's Hospital, Cincinnati
Wangensteen, Owen H.	Univ. of Minnesota
Warren, Shields	Harvard Med.
Wasteneys, H.	Univ. of Toronto
Wearn, J. T.	Lakeside Hospital, Cleveland, Ohio
Weber, Clarence J.	Univ. of Kansas Med.
Webster, Bruce	Cornell Univ. Medical Coll.
Webster, J. P.	Presbyterian Hospital, N. Y. City
Webster, Leslie T.	Rockefeller Institute
Wedd, Alfred M.	Clifton Springs Sanitarium
Weech, A. A.	Coll. Physicians and Surgeons
Weil, Arthur	Northwestern Univ.
Weiskotten, Herman G.	Syracuse Univ.
Weiss, Charles	Mt. Zion Hospital, San Francisco
Weiss, Emil	Univ. of Illinois Med.
Weiss, Harry	Columbia Univ.
Weiss, Paul A.	Univ. of Chicago
Weiss, Soma	Boston City Hospital
Weld, Julia T.	Columbia Univ.
Welker, W. H.	Univ. of Illinois Medical Coll.
Wen, I. C.	Peiping Union Medical Coll.
Wenner, William F.	Washington Univ. Med.
Werkman, Chester H.	Iowa State Coll.
West, Edward S.	Univ. of Oregon Med.
West, Randolph	Presbyterian Hospital, N. Y. City
Wetzel, Norman C.	Western Reserve Univ.
Weymouth, Frank W.	Stanford Univ.
Wheeler, Mary W.	N. Y. State Dept. of Health
Whipple, George H.	Univ. of Rochester
Whitaker, D. M.	Stanford Univ.
White, Abraham	Yale Univ.
White, H. L.	Washington Univ.
Wible, Charles L.	Univ. of Nebraska
Wiebe, A. H.	Huntsville, Texas
Wiggers, Carl J.	Western Reserve Univ.
Wilhelmj, C. M.	Creighton Univ. Med.
Williams, Horatio B.	Columbia Univ.
Williams, J. R.	Highland Hospital, Rochester, N. Y.
Williams, John W.	Massachusetts Institute of Technology
Williams, Robert R.	Western Electric Company
Willier, Benjamin H.	Univ. of Rochester
Willis, Henry S.	Maybury Sanitarium, Northville, Mich.
Wilson, D. Wright	Univ. of Pennsylvania
Wilson, Frank N.	Univ. of Michigan
Wilson, James R.	Syracuse, N. Y.
Winslow, C.-E. A.	Yale Univ.
Winternitz, Milton C.	Yale Univ.
Wiseman, B. K.	Ohio State Univ.

Witebsky, E.	Beth Israel Hospital, N. Y. City
Witschi, Emil	State Univ. of Iowa
Witzemann, Edgar J.	Univ. of Wisconsin
Woglom, W. H.	Columbia Univ.
Wolbach, S. B.	Harvard Univ.
Wolfe, J. M.	Vanderbilt Univ.
Wood, Francis C.	Columbia Univ.
Woodruff, L. L.	Yale Univ.
Wright, Harold N.	Univ. of Minnesota
Wright, Irving S.	Cornell Univ. Medical Coll.
Wu, Hsien	Peiping Union Medical Coll., China
Wulzen, Rosalind	Oregon State Coll.
Wyckoff, R. W. G.	Rockefeller Institute

Yatsu, Naohide	Zoological Institute, Tokyo, Japan
Yeager, J. F.	Iowa State Coll.
Yerkes, Robert M.	Yale Univ.
Youland, William E., Jr.	N. Y. Homeopathic Medical Coll.
Young, C. C.	Dept. of Health, Mich.
Young, William G.	Univ. of California, Los Angeles
Youngburg, Guy E.	Univ. of Buffalo Med.
Yudkin, Arthur M.	Yale Univ.

Zerfas, L. G.	Indianapolis City Hospital
Zia, S. H.	Peiping Union Medical Coll.
Zimmerman, Harry M.	Yale Univ.
Zinsser, Hans	Harvard Univ.
ZoBell, Claude E.	Scripps Institution, La Jolla, Calif.
Zozaya, José	Gladwyne, Pa.
Zucker, Theodore F.	Columbia Univ.